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FILE 'USPATFULL' ENTERED AT 18:34:51 ON 29 DEC 2002
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FILE 'BIOSIS' ENTERED AT 18:34:51 ON 29 DEC 2002
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=> s hiv and tar
L4        3924 HIV AND TAR

=> s l4 and snorna
L5           1 L4 AND SNORNA

=> d l5 ibib abs tot

L5   ANSWER 1 OF 1  USPATFULL
ACCESSION NUMBER:        1999:81758  USPATFULL
TITLE:                   Non-activated receptor complex proteins and uses
                         thereof
INVENTOR(S):             Davis, Roger J., Princeton, MA, United States
                         Galcheva-Gargova, Zoya, Worcester, MA, United States
PATENT ASSIGNEE(S):      University of Massachusetts, Boston, MA, United States
                         (U.S. corporation)

                              NUMBER        KIND    DATE
                         ---------------- ------ --------
PATENT INFORMATION:      US 5925566               19990720
APPLICATION INFO.:       US 1997-870518           19970606  (8)

                                NUMBER         DATE
                         ------------------ --------
PRIORITY INFORMATION:    US 1996-19219P     19960606 (60)
DOCUMENT TYPE:           Utility
FILE SEGMENT:            Granted
PRIMARY EXAMINER:        Campell, Bruce R.
ASSISTANT EXAMINER:      Nguyen, Dave Trong
LEGAL REPRESENTATIVE:    Fish & Richardson, P.C.
NUMBER OF CLAIMS:        23
EXEMPLARY CLAIM:         1
NUMBER OF DRAWINGS:      22 Drawing Figure(s); 18 Drawing Page(s)
LINE COUNT:              2438
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB       The invention features a substantially pure ZPR1 polypeptide. For
         example, a ZPR1 polypeptide that specifically binds to a non-activated
         membrane-bound receptor (e.g., EGF or PDGF receptors) and specifically
         binds small nucleolar RNAs (e.g., U3). ZPR1 polypeptides can be isolated
         from any eukaryote, including mammals (e.g. rodents and humans) and
         fungi (e.g., S. cerevisiae and S. pombe).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> d kwic

L5   ANSWER 1 OF 1  USPATFULL
SUMM     "Small nucleolar RNA" or "  ***snoRNA***  " refers to RNA sequences
         located in the nucleolus and includes the sequences encoded by the U3,
         U10, U15, U22, and.  .  .
DETD     .  .  .  The interaction of ZPR1 with the snoRNAs U3, U10, U15, U22,
         U33, or the viral RNAs corresponding to the RRE (  ***HIV***   Rev
         responsive element),   ***HIV***   trans-acting response element (
           ***TAR***  ), and adenovirus VA-RNA1 (Akusjavari et al., Proc. Natl.
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  .     (1988); Bartel.  .  .  was investigated by this method. No binding of
        ZPR1 was detected in experiments using viral RNA corresponding to RRE,
        the   ***TAR***   , or adenovirus VA-RNA1. In contrast, binding to ZPR1
        was detected in experiments using several small nucleolar RNAs
        (snoRNAs), including U3,.  .  .
DETD    The specificity of the interaction of RNA with ZPR1 was examined in
        greater detail in competition experiments using the   ***snoRNA***   ,
        U3. A [.sup.32 P]-labeled U3 probe was incubated with ZPR1 alone or in
        the presence of excess non-radioactive U3, anti-sense.  .  .


=>
=> d history


     FILE 'MEDLINE, CAPLUS, LIFESCI, EMBASE, USPATFULL, BIOSIS' ENTERED AT
     18:34:51 ON 29 DEC 2002
L4          3924 S HIV AND TAR
L5             1 S L4 AND SNORNA

=> s l4 and u16
L6             1 L4 AND U16

=> s l6 not l5
L7             1 L6 NOT L5

=> d l7 ibib abs tot

L7   ANSWER 1 OF 1  USPATFULL
ACCESSION NUMBER:         2001:142079  USPATFULL
TITLE:                    Method for screening nucleic acid catalysts
INVENTOR(S):              Burgin, Alex, Chula Vista, CA, United States
                          Beigelman, Leonid, Longmont, CO, United States
                          Bellon, Laurent, Boulder, CO, United States
PATENT ASSIGNEE(S):       Ribozyme Pharmaceuticals, Inc., Boulder, CO, United
                          States (U.S. corporation)

                               NUMBER         KIND    DATE
                          ---------------- ------ --------
PATENT INFORMATION:       US 6280936          B1   20010828
APPLICATION INFO.:        US 1998-94381            19980609  (9)

                                 NUMBER         DATE
                          ------------------- --------
PRIORITY INFORMATION:     US 1997-49002P      19970609 (60)
DOCUMENT TYPE:            Utility
FILE SEGMENT:             GRANTED
PRIMARY EXAMINER:         Fredman, Jeffrey
LEGAL REPRESENTATIVE:     McDonnell Boehnen Hulbert & Berghoff
NUMBER OF CLAIMS:         24
EXEMPLARY CLAIM:          1
NUMBER OF DRAWINGS:       23 Drawing Figure(s); 23 Drawing Page(s)
LINE COUNT:               2403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      Nucleic acid catalysts, method of screening for variants of nucleic acid
        catalysts, synthesis of ribozyme libraries and discovery of gene
        sequences are described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> d kwic

L7   ANSWER 1 OF 1  USPATFULL
DETD    In yet another embodiment, the nucleotide linker (L) is a nucleic acid
        aptamer, such as an ATP aptamer,   ***HIV***   Rev aptamer (RRE),
          ***HIV***   Tat aptamer (  ***TAR***  ) and others (for a review see
        Gold et al., 1995, Annu. Rev. Biochem., 64, 763; and Szostak &
        Ellington, 1993,.  .  .
DETD    .  .  .  that for the hammerhead ribozyme one residue (A15.1) must
        remain constant; A15.1 forms a base pair with a substrate nucleotide (
          ***U16***  .1) but is also absolutely required for ribozyme activity. It
        is the only residue within the hammerhead ribozyme that is part.  .  .
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E1     .      2     MICHIELUTTI RONDA E/AU
E2            5     MICHIELUTTI THOMAS J/AU
E3            0 --> MICHIENZI ?/AU
E4           27     MICHIENZI A/AU
E5           21     MICHIENZI ALESSANDRO/AU
E6            2     MICHIENZI F/AU
E7            3     MICHIENZI GIACOMO F/AU
E8            6     MICHIENZI K E/AU
E9            1     MICHIENZI KATHLEEN E/AU
E10           3     MICHIENZI L J/AU
E11          15     MICHIENZI M/AU
E12           1     MICHIENZI M A/AU

=> s e4-5
L8           48 ("MICHIENZI A"/AU OR "MICHIENZI ALESSANDRO"/AU)

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9           15 DUP REM L8 (33 DUPLICATES REMOVED)

=> d l9 ibib abs tot

L9   ANSWER 1 OF 15     MEDLINE                          DUPLICATE 1
ACCESSION NUMBER:    2002647867     MEDLINE
DOCUMENT NUMBER:     22294961   PubMed ID: 12376617
TITLE:               A nucleolar TAR decoy inhibitor of HIV-1 replication.
AUTHOR:                ***Michienzi Alessandro***  ; Li Shirley; Zaia John A;
                     Rossi John J
CORPORATE SOURCE:    Divisions of Molecular Biology and Virology, Beckman
                     Research Institute of the City of Hope, 1450 East Duarte
                     Road, Duarte, CA 91010-3011, USA.
CONTRACT NUMBER:     AI29329 (NIAID)
     AI46030 (NIAID)
SOURCE:              PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE
                     UNITED STATES OF AMERICA, (2002 Oct 29) 99 (22) 14047-52.
                     Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200212
ENTRY DATE:          Entered STN: 20021031
                     Last Updated on STN: 20021217
                     Entered Medline: 20021209
AB   Tat is a critical regulatory factor in HIV-1 gene expression. It mediates
     the transactivation of transcription from the HIV-1 LTR by binding to the
     transactivation response (TAR) element in a complex with cyclin T1.
     Because of its critical and early role in HIV gene expression, Tat and its
     interaction with the TAR element constitute important therapeutic targets
     for the treatment of HIV-1 infection. Based on the known nucleolar
     localization properties of Tat, we constructed a chimeric small nucleolar
     RNA-TAR decoy that localizes to the nucleoli of human cells and
     colocalizes in the nucleolus with a Tat-enhanced GFP fusion protein. When
     the chimeric RNA was stably expressed in human T lymphoblastoid CEM cells
     it potently inhibited HIV-1 replication. These results demonstrate that
     the nucleolar trafficking of Tat is critical for HIV-1 replication and
     suggests a role for the nucleolus in HIV-1 viral replication.

L9   ANSWER 2 OF 15  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:        2002:899156  CAPLUS
TITLE:                   Intracellular ribozyme applications
AUTHOR(S):               Castanotto, D.; Li, J. R.;   ***Michienzi, A.***  ;
                         Langlois, M.-A.; Lee, N. S.; Puymirat, J.; Rossi, J.
                         J.
CORPORATE SOURCE:        Division of Molecular Biology, Beckman Research
                         Institute of the City of Hope, Duarte, CA, 91010-3011
SOURCE:                  Biochemical Society Transactions (2002), 30(6),
                         1140-1145
                         CODEN: BCSTB5; ISSN: 0300-5127
PUBLISHER:               Portland Press Ltd.
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   The exquisite target selectivity of trans -acting ribozymes has fostered
     their use as potential therapeutic agents and tools for down-regulating
     cellular transcripts.  In living cells, free diffusion of RNAs is
     extremely limited, if it exists at all.  Thus, getting ribozymes to
```

transcript with the target RNA. In addn., not all sites along a given target RNA are equally accessible to ribozyme base pairing. Cellular proteins greatly influence the trafficking and structure of RNA, and therefore making ribozymes work effectively in cells a significant challenge. This article addresses the problems of getting engineered ribozymes to effectively pair with and cleave targets in cells. The work described here illuminates methods for target-site selection on native mRNAs, methods for ribozyme expression, and strategies for obtaining a discrete intracellular localization of ribozymes.

L9 ANSWER 3 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:624770 BIOSIS
DOCUMENT NUMBER: PREV200200624770
TITLE: Anti-HIV therapy: New concepts & results retroviral delivery of combinations of anti-HIV RNAs in cord blood CD34+ and HOS-CCR5/CD4 cells.
AUTHOR(S): Kleihauer, Annette (1); Kim, James (1); Bauer, Gerhard (1); Cagnon, Laurance (1); ***Michienzi, Alessandro (1)*** ; Zaia, John A. (1); Rossi, John J. (1)
CORPORATE SOURCE: (1) City of Hope National Medical Center/Beckman Research Institute, Duarte, CA, 91010 USA
SOURCE: Journal of Human Virology, (January February, 2002) Vol. 5, No. 1, pp. 89-90. http://www.humanvirology.com/. print. Meeting Info.: 2002 International Meeting of the Institute of Human Virology Baltimore, Maryland, USA September 09-13, 2002 Institute of Human Virology . ISSN: 1090-9508.
DOCUMENT TYPE: Conference
LANGUAGE: English

L9 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:872767 CAPLUS
TITLE: Chimeric snoRNA-Rev binding aptamer RNA as Rev protein decoy and inhibitor of HIV1 replication
INVENTOR(S): Bozzoni, Irene; ***Michienzi, Alessandro*** ; Buonomo, Sara Cristina Barbara
PATENT ASSIGNEE(S): Universita' Degli Studi Di Roma 'la Sapienza', Italy
SOURCE: Ital., 35 pp.
CODEN: ITXXBY
DOCUMENT TYPE: Patent
LANGUAGE: Italian
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IT 1305300	B1	20010504	IT 1999-RM126	19990224

PRIORITY APPLN. INFO.: IT 1999-RM126 19990224
AB A chimeric RNA mol. which functions as a decoy for HIV1 Rev protein is disclosed. This chimeric RNA consists of an snoRNA fragment, which is responsible for translocation of the chimera to the nucleolus, and a Rev protein-binding RNA fragment. Thus, a construct encoding an snoRNA U16 fragment fused to the Rev binding element of RRE was prepd. In recombinant 293 cells the chimeric RNA was found in the nucleus where it bound to Rev protein and was exported to the cytoplasm.

L9 ANSWER 5 OF 15 MEDLINE
ACCESSION NUMBER: 2002680695 IN-PROCESS
DOCUMENT NUMBER: 22328739 PubMed ID: 12440991
TITLE: Intracellular ribozyme applications.
AUTHOR: Castanotto D; Li J R; ***Michienzi A*** ; Langlois M A; Lee N S; Puymirat J; Rossi J J
CORPORATE SOURCE: Division of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA 91010-3011, U.S.A.
SOURCE: BIOCHEMICAL SOCIETY TRANSACTIONS, (2001 Dec) 30 (6) 1140-5. Journal code: 7506897. ISSN: 0300-5127.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20021121
Last Updated on STN: 20021213
AB The exquisite target selectivity of trans -acting ribozymes has fostered their use as potential therapeutic agents and tools for down-regulating cellular transcripts. In living cells, free diffusion of RNAs is extremely limited, if it exists at all. Thus, getting ribozymes to base-pair with

the target RNA. In addition, not all sites along a given target RNA are equally accessible to ribozyme base pairing. Cellular proteins greatly influence the trafficking and structure of RNA, and therefore making ribozymes work effectively in cells a significant challenge. This article addresses the problems of getting engineered ribozymes to effectively pair with and cleave targets in cells. The work described here illuminates methods for target-site selection on native mRNAs, methods for ribozyme expression, and strategies for obtaining a discrete intracellular localization of ribozymes.

L9 ANSWER 6 OF 15 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2001535732 MEDLINE
DOCUMENT NUMBER: 21467394 PubMed ID: 11582807
TITLE: Intracellular applications of ribozymes.
AUTHOR: ***Michienzi A*** ; Rossi J J
CORPORATE SOURCE: Molecular Biology Department, Beckman Research Institute of the City of Hope, Duarte, California 91010, USA.
CONTRACT NUMBER: AI 29329 (NIAID)
AI 42552 (NIAID)
AI 46030 (NIAID)
SOURCE: METHODS IN ENZYMOLOGY, (2001) 341 581-96.
Journal code: 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20011004
Last Updated on STN: 20020308
Entered Medline: 20020307

L9 ANSWER 7 OF 15 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000422579 MEDLINE
DOCUMENT NUMBER: 20381318 PubMed ID: 10922055
TITLE: Ribozyme-mediated inhibition of HIV 1 suggests nucleolar trafficking of HIV-1 RNA.
AUTHOR: ***Michienzi A*** ; Cagnon L; Bahner I; Rossi J J
CORPORATE SOURCE: Department of Molecular Biology, Beckman Research Institute of the City of Hope, and Graduate School of Biological Sciences, City of Hope, Duarte, CA 91010-3011, USA.
CONTRACT NUMBER: 5 F32 GM18898-02 (NIGMS)
AI29329 (NIAID)
AI42552 (NIAID)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2000 Aug 1) 97 (16) 8955-60.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000915
Last Updated on STN: 20000915
Entered Medline: 20000905

AB The HIV regulatory proteins Tat and Rev have a nucleolar localization property in human cells. However, no functional role has been attributed to this localization. Recently it has been demonstrated that expression of Rev induces nucleolar relocalization of some protein factors involved in Rev export. Because the function of Rev is to bind HIV RNA and facilitate transport of singly spliced and unspliced RNA to the cytoplasm, it is likely that the nucleolus plays a critical role in HIV-1 RNA export. As a test for trafficking of HIV-1 RNAs into the nucleolus, a hammerhead ribozyme that specifically cleaves HIV-1 RNA was inserted into the body of the U16 small nucleolar RNA, resulting in accumulation of the ribozyme within the nucleoli of human cells. HeLa CD4(+) and T cells expressing this nucleolar localized ribozyme exhibit dramatically suppressed HIV-1 replication. The results presented here suggest a trafficking of HIV-1 RNA through the nucleoli of human cells, thus posing a different paradigm for lentiviral RNA processing.

L9 ANSWER 8 OF 15 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999373046 MEDLINE
DOCUMENT NUMBER: 99373046 PubMed ID: 10445874
TITLE: The Rev protein is able to transport to the cytoplasm small nucleolar RNAs containing a Rev binding element.
AUTHOR: Buonomo S B; ***Michienzi A*** ; De Angelis F G; Bozzoni

CORPORATE SOURCE: Istituto Pasteur, Fondazione Cenci-Bolognetti, Dipartimento di Genetica e Biologia Molecolare, Universita di Roma La Sapienza, Italy.
SOURCE: RNA, (1999 Aug) 5 (8) 993-1002.
Journal code: 9509184. ISSN: 1355-8382.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990913
Last Updated on STN: 19990913
Entered Medline: 19990830

AB Small nucleolar RNAs (snoRNAs) were utilized to express Rev-binding sequences inside the nucleolus and to test whether they are substrates for Rev binding and transport. We show that U16 snoRNA containing the minimal binding site for Rev stably accumulates inside the nucleolus maintaining the interaction with the basic C/D snoRNA-specific factors. Upon Rev expression, the chimeric RNA is exported to the cytoplasm, where it remains bound to Rev in a particle devoid of snoRNP-specific factors. These data indicate that Rev can elicit the functions of RNA binding and transport inside the nucleolus.

L9 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:108061 CAPLUS
DOCUMENT NUMBER: 132:248431
TITLE: A chimeric nucleolar Rev decoy inhibits the HIV replication
AUTHOR(S): ***Michienzi, A.*** ; Cagnon, L.; Bozzoni, I.; Rossi, J. J.
CORPORATE SOURCE: Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA, 91010, USA
SOURCE: Nucleic Acids Symposium Series (1999), 41(Symposium on RNA Biology III: RNA, Tool & Target), 211-214
CODEN: NACSD8; ISSN: 0261-3166
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In order to investigate the role of the nucleolus in Rev function, the authors constructed a nucleolar Rev decoy. They used the RBE (Rev binding element) sequence of the HIV genome as a decoy and U16snoRNA as a vector to deliver the decoy into the nucleolar compartment (clone U16RBE). They then analyzed the U16RBE expression and anti-HIV activity in a human cell line. The data show that a nucleolar decoy is able to suppress the HIV replication, and suggest that the nucleolus can play a crit. role in Rev function.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 10 OF 15 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998211332 MEDLINE
DOCUMENT NUMBER: 98211332 PubMed ID: 9551610
TITLE: Inhibition of human immunodeficiency virus type 1 replication by nuclear chimeric anti-HIV ribozymes in a human T lymphoblastoid cell line.
AUTHOR: ***Michienzi A*** ; Conti L; Varano B; Prislei S; Gessani S; Bozzoni I
CORPORATE SOURCE: Istituto Pasteur, Fondazione Cenci-Bolognetti, Department of Genetics and Molecular Biology, University La Sapienza, Rome, Italy.
SOURCE: HUMAN GENE THERAPY, (1998 Mar 20) 9 (5) 621-8.
Journal code: 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980611
Last Updated on STN: 19980611
Entered Medline: 19980529

AB Human immunodeficiency virus (HIV) infection represents one of the most challenging systems for gene therapy. Thanks to the extended knowledge of the molecular biology of the HIV life cycle, many different strategies have been developed including transdominant modifications of HIV proteins, RNA decoys, antisense RNA, ribozymes, and intracellular antibody fragments. In this paper, we have tested in a human T lymphoblastoid cell

co-localize inside the nucleus with the Rev pre-mRNA before it is spliced and transported to the cytoplasm. This result was obtained by inserting the ribozyme in the spliceosomal U1 small nuclear RNA (snRNA) and in a derivative that has perfect complementarity with the 5' splice site of the Rev pre-mRNA. These ribozymes were tested in human T cell clones and were shown to be very efficient in inhibiting viral replication. Not only were the p24 levels in the culture medium drastically reduced but so were the intracellular HIV transcripts. Control disabled ribozymes enabled us to show the specificity of the ribozyme activity. Therefore, these constructs have potential utility for gene therapy of HIV-1 infection.

L9 ANSWER 11 OF 15 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97316827 MEDLINE
DOCUMENT NUMBER: 97316827 PubMed ID: 9174101
TITLE: Use of adenoviral VAI small RNA as a carrier for cytoplasmic delivery of ribozymes.
AUTHOR: Prislei S; Buonomo S B; ***Michienzi A*** ; Bozzoni I
CORPORATE SOURCE: Istituto Pasteur, Fondazione Cenci-Bolognetti, Dipartimento di Genetica e Biologia Molecolare, Universita La Sapienza, Roma, Italy.
SOURCE: RNA, (1997 Jun) 3 (6) 677-87.
Journal code: 9509184. ISSN: 1355-8382.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970709
Last Updated on STN: 19970709
Entered Medline: 19970624

AB The in vivo effectiveness of therapeutic RNAs, like antisense molecules and ribozymes, relies on several features: RNA molecules need to be expressed at high levels in the correct cellular compartment as stable and active molecules. The exploitation of "natural" small RNA coding genes as expressing cassettes gives high chances to fulfill these requirements. We have investigated the utilization of the adenoviral VAI RNA as a cytoplasmatic carrier for expressing ribozymes against HIV-1. The conserved 5' leader sequence of HIV was chosen as a target, because it is present in all the viral transcripts and is highly conserved. Hammerhead ribozymes were substituted to different portions of the VAI RNA and the resulting chimera were tested in the in vivo system of Xenopus laevis oocytes for their level of accumulation, cellular compartmentalization, and assembly in specific ribonucleoparticles containing the La antigen. Interesting differences in the activity of the different chimera were found in both in vitro cleavage assays and S100 extracts of injected oocytes where the catalytic activity of the ribozymes in the RNP context can be analyzed.

L9 ANSWER 12 OF 15 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 96293503 MEDLINE
DOCUMENT NUMBER: 96293503 PubMed ID: 8692972
TITLE: U1 small nuclear RNA chimeric ribozymes with substrate specificity for the Rev pre-mRNA of human immunodeficiency virus.
AUTHOR: ***Michienzi A*** ; Prislei S; Bozzoni I
CORPORATE SOURCE: Istituto Pasteur Fondazione Cenci-Bolognetti, Rome, Italy.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jul 9) 93 (14) 7219-24.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960911
Last Updated on STN: 19970203
Entered Medline: 19960829

AB The in vivo effectiveness of ribozymes strongly depends on the correct choice of the vector molecule. High levels of expression, stability, active conformation, and correct cellular localization are the most important features for a ribozyme vector. We have exploited the utilization of the U1 small nuclear RNA (snRNA) as a vector for specifically targeting a ribozyme into the nucleus. The Rev pre-mRNA of human immunodeficiency virus type 1 was chosen as target for testing the activity of the Ul-ribozyme. The catalytic core of the hammerhead motif, plus the recognition sequences, substituted the stem-loop III of the U1

vitro. In addition, in the in vivo system of Xenopus laevis oocytes, the U1-chimeric ribozyme accumulates in large amounts in the nucleus and produces a considerable reduction of Rev pre-mRNA levels. The Rev-specific ribozyme was also inserted in a derivative of the U1 snRNA mutated in the region of pairing with the 5' splice site, such as to match it with the suboptimal splice junction of the Rev precursor. This construct shows more efficient reduction of Rev pre-mRNA in vivo than the wild-type U1 vector.

L9 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:281947 BIOSIS
DOCUMENT NUMBER: PREV199497294947
TITLE: Two novel small nucleolar RNA U16 and U18 are encoded inside introns of the same ribosomal protein gene.
AUTHOR(S): Fragapane, Paola; Prislei, Silvia; ***Michienzi,*** ***Alessandro*** ; Presutti, Carlo; Caffarelli, Elisa; Bozzoni, Irene
CORPORATE SOURCE: Centro Acidi Nucleici C.N.R., Dip. Genetica e Biol. Molecolare, Univ. "La Sapienza", Rome Italy
SOURCE: Journal of Cellular Biochemistry Supplement, (1994) Vol. 0, No. 18C, pp. 113.
Meeting Info.: Keystone Symposium on the Eukaryotic Nucleus Tamarron, Colorado, USA February 13-20, 1994
ISSN: 0733-1959.
DOCUMENT TYPE: Conference
LANGUAGE: English

L9 ANSWER 14 OF 15 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 94119676 MEDLINE
DOCUMENT NUMBER: 94119676 PubMed ID: 7507233
TITLE: Two different snoRNAs are encoded in introns of amphibian and human L1 ribosomal protein genes.
AUTHOR: Prislei S; ***Michienzi A*** ; Presutti C; Fragapane P; Bozzoni I
CORPORATE SOURCE: Istituto Pasteur Fondazione Cenci-Bolognetti, Rome, Italy.
SOURCE: NUCLEIC ACIDS RESEARCH, (1993 Dec 25) 21 (25) 5824-30.
Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X75486; GENBANK-X75487; GENBANK-X75488
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940312
Last Updated on STN: 19960129
Entered Medline: 19940222
AB We previously reported that the third intron of the X.laevis L1 ribosomal protein gene encodes for a snoRNA called U16. Here we show that four different introns of the same gene contain another previously uncharacterized snoRNA (U18) which is associated with fibrillarin in the nucleolus and which originates by processing of the pre-mRNA. The pathway of U18 RNA release from the pre-mRNA is the same as the one described for U16: primary endonucleolytic cleavages upstream and downstream of the U18 coding region produce a pre-U18 RNA which is subsequently trimmed to the mature form. Both the gene organization and processing of U18 are conserved in the corresponding genes of X.tropicalis and H.sapiens. The L1 gene thus has a composite structure, highly conserved in evolution, in which sequences coding for a ribosomal protein are intermingled with sequences coding for two different snoRNAs. The nucleolar localization of these different components suggests some common function on ribosome biosynthesis.

L9 ANSWER 15 OF 15 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 93327782 MEDLINE
DOCUMENT NUMBER: 93327782 PubMed ID: 8335006
TITLE: A novel small nucleolar RNA (U16) is encoded inside a ribosomal protein intron and originates by processing of the pre-mRNA.
AUTHOR: Fragapane P; Prislei S; ***Michienzi A*** ; Caffarelli E; Bozzoni I
CORPORATE SOURCE: Centro Acidi Nucleici of CNR, Universita La Sapienza, Roma, Italy.
SOURCE: EMBO JOURNAL, (1993 Jul) 12 (7) 2921-8.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

OTHER SOURCE: GENBANK-X72205
ENTRY MONTH: 199308
ENTRY DATE: Entered STN: 19930903
Last Updated on STN: 19930903
Entered Medline: 19930820

AB We report that the third intron of the L1 ribosomal protein gene of Xenopus laevis encodes a previously uncharacterized small nucleolar RNA that we called U16. This snRNA is not independently transcribed; instead it originates by processing of the pre-mRNA in which it is contained. Its sequence, localization and biosynthesis are phylogenetically conserved: in the corresponding intron of the human L1 ribosomal protein gene a highly homologous region is found which can be released from the pre-mRNA by a mechanism similar to that described for the amphibian U16 RNA. The presence of a snoRNA inside an intron of the L1 ribosomal protein gene and the phylogenetic conservation of this gene arrangement suggest an important regulatory/functional link between these two components.

=> s l9 and tar
L10 1 L9 AND TAR

=> d l10 ibib abs tot

L10 ANSWER 1 OF 1 MEDLINE
ACCESSION NUMBER: 2002647867 MEDLINE
DOCUMENT NUMBER: 22294961 PubMed ID: 12376617
TITLE: A nucleolar ***TAR*** decoy inhibitor of HIV-1 replication.
AUTHOR: ***Michienzi Alessandro*** ; Li Shirley; Zaia John A; Rossi John J
CORPORATE SOURCE: Divisions of Molecular Biology and Virology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010-3011, USA.
CONTRACT NUMBER: AI29329 (NIAID)
AI46030 (NIAID)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2002 Oct 29) 99 (22) 14047-52. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20021031
Last Updated on STN: 20021217
Entered Medline: 20021209

AB Tat is a critical regulatory factor in HIV-1 gene expression. It mediates the transactivation of transcription from the HIV-1 LTR by binding to the transactivation response (***TAR***) element in a complex with cyclin T1. Because of its critical and early role in HIV gene expression, Tat and its interaction with the ***TAR*** element constitute important therapeutic targets for the treatment of HIV-1 infection. Based on the known nucleolar localization properties of Tat, we constructed a chimeric small nucleolar RNA- ***TAR*** decoy that localizes to the nucleoli of human cells and colocalizes in the nucleolus with a Tat-enhanced GFP fusion protein. When the chimeric RNA was stably expressed in human T lymphoblastoid CEM cells it potently inhibited HIV-1 replication. These results demonstrate that the nucleolar trafficking of Tat is critical for HIV-1 replication and suggests a role for the nucleolus in HIV-1 viral replication.

=> s tar and nucleoli
L11 38 TAR AND NUCLEOLI

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12 21 DUP REM L11 (17 DUPLICATES REMOVED)

=> s l12 not l10
L13 20 L12 NOT L10

=> d l13 ibib abs tot

L13 ANSWER 1 OF 20 MEDLINE
ACCESSION NUMBER: 97375656 MEDLINE

TITLE: . Xlrbpa, a double-stranded RNA-binding protein associated with ribosomes and heterogeneous nuclear RNPs.
AUTHOR: Eckmann C R; Jantsch M F
CORPORATE SOURCE: Department of Cytology and Genetics, Institute of Botany, University of Vienna, A-1030 Vienna, Austria.
SOURCE: JOURNAL OF CELL BIOLOGY, (1997 Jul 28) 138 (2) 239-53. Journal code: 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970908
Last Updated on STN: 19970908
Entered Medline: 19970825

AB we have cloned and characterized Xlrbpa, a double-stranded RNA-binding protein from Xenopus laevis. Xlrbpa is a protein of 33 kD and contains three tandemly arranged, double-stranded RNA-binding domains (dsRBDs) that bind exclusively to double-stranded RNA in vitro, but fail to bind either single-stranded RNA or DNA. Sequence data and the overall organization of the protein suggest that Xlrbpa is the Xenopus homologue of human ***TAR*** -RNA binding protein (TRBP), a protein isolated by its ability to bind to human immunodeficiency virus (HIV) ***TAR*** -RNA. In transfection assays, TRBP has also been shown to inhibit the interferon-induced protein kinase PKR possibly by direct physical interaction. To determine the function of Xlrbpa and its human homologue we studied the expression and intracellular distribution of the two proteins. Xlrbpa is ubiquitously expressed with marked quantitative differences amongst all tissues. Xlrbpa and human TRBP can be detected in the cytoplasm and nucleus by immunofluorescence staining and Western blotting. Sedimentation gradient analyses and immunoprecipitation experiments suggest an association of cytoplasmic Xlrbpa with ribosomes. In contrast, a control construct containing two dsRBDs fails to associate with ribosomes in microinjected Xenopus oocytes. Nuclear staining of Xenopus lampbrush chromosome preparations showed the association of the protein with ***nucleoli*** , again indicating an association of the protein with ribosomal RNAs. Additionally, Xlrbpa could be located on lampbrush chromosomes and in snurposomes. Immunoprecipitations of nuclear extracts demonstrated the presence of the protein in heterogeneous nuclear (hn) RNP particles, but not in small nuclear RNPs, explaining the chromosomal localization of the protein. It thus appears that Xlrbpa is a general double-stranded RNA-binding protein which is associated with the majority of cellular RNAs, ribosomal RNAs, and hnRNAs either alone or as part of an hnRNP complex.

L13 ANSWER 2 OF 20 MEDLINE
ACCESSION NUMBER: 97360029 MEDLINE
DOCUMENT NUMBER: 97360029 PubMed ID: 9217057
TITLE: Bovine immunodeficiency virus tat gene: cloning of two distinct cDNAs and identification, characterization, and immunolocalization of the tat gene products.
AUTHOR: Fong S E; Greenwood J D; Williamson J C; Derse D; Pallansch L A; Copeland T; Rasmussen L; Mentzer A; Nagashima K; Tobin G; Gonda M A
CORPORATE SOURCE: Laboratory of Cell and Molecular Structure, SAIC Frederick, NCI-Frederick Cancer Research and Development Center, Maryland 21702-1201, USA.. Fong@mail.ncifcrf.gov
SOURCE: VIROLOGY, (1997 Jul 7) 233 (2) 339-57. Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970825
Last Updated on STN: 19970825
Entered Medline: 19970813

AB cDNAs encoding the bovine immunodeficiency virus (BIV) transactivator gene (tat) were cloned from virally infected cells and characterized. BIV expresses two distinct tat mRNAs composed of three exons that are derived by alternative splicing. The BIV tat mRNA splice variants encode Tat proteins of 103 (Tat103) and 108 (Tat108) amino acids. The Tat103 coding region is specified only by exon 2, while that of Tat108 is specified by a truncated exon 2 and the first 30 nt of exon 3. Thus, the first 98 amino acids of each Tat are identical, and have amino terminal, cysteine-rich, conserved core, basic, and carboxyl-terminal domains similar to Tats

14-kDa phosphorylated Tat protein identical in size to recombinant Tat expressed in bacteria. BIV Tat was shown to localize exclusively in the ***nucleoli*** of virally infected and Tat-expressing cells. Reporter gene assays indicated that Tat103 and Tat108 can strongly transactivate the BIV long terminal repeat (LTR) in virally permissive canine Cf2Th and nonpermissive HeLa and mouse NIH 3T3 cells, but not in permissive lapine EREp cells. However, an intact BIV tat gene is required for viral replication in both Cf2Th and EREp cells. Strong LTR activation by BIV Tat requires a ***TAR*** (transactivation responsive) element delimited by viral nt +1 to +31 and the Tat basic domain. BIV Tat strongly cross-transactivates the HIV-1 LTR in a ***TAR*** -dependent manner in Cf2Th, but not in EREp, HeLa, or NIH 3T3 cells. In contrast, strong, ***TAR*** -dependent cross-transactivation of the BIV LTR by HIV-1 Tat could not be demonstrated in any of these cell types. In Cf2Th cells Tat108 effects a moderately stronger transactivation of the BIV LTR than Tat103, indicative of a functional difference in BIV Tat proteins encoded by the mRNA splice variants. The present studies demonstrate that BIV Tat parallels the primate lentiviral Tats in structure and biochemistry but is not interchangeable with the latter.

L13 ANSWER 3 OF 20 MEDLINE
ACCESSION NUMBER: 91175688 MEDLINE
DOCUMENT NUMBER: 91175688 PubMed ID: 2078551
TITLE: Regulation of expression of human immunodeficiency virus.
AUTHOR: Pavlakis G N; Felber B K
CORPORATE SOURCE: National Cancer Institute, Frederick Cancer Research Facility, BRI-Basic Research Program, MD 21701-1013.
CONTRACT NUMBER: N01-CO-74101 (NCI)
SOURCE: NEW BIOLOGIST, (1990 Jan) 2 (1) 20-31. Ref: 141
Journal code: 9000976. ISSN: 1043-4674.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910519
Last Updated on STN: 19970203
Entered Medline: 19910501

AB Human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), encodes regulatory factors necessary for its expression. The study of these factors clearly indicates the importance and complexity of post-trascriptional processes in regulating gene expression. Two regulatory proteins, Tat and Rev, are necessary for viral replication. These small nuclear proteins accumulate primarily in the ***nucleoli*** and act on HIV via sequence-specific elements found on the viral RNA. Both the Tat responsive element (***TAR***) and the Rev responsive element (RRE) map within regions of strong RNA secondary structure. While all viral mRNAs contain ***TAR*** , only the mRNAs producing HIV structural proteins contain RRE. Tat increases the levels of all viral mRNAs. Its function is complex and involves transcriptional and possibly post-transcriptional steps. Rev promotes the transport of viral mRNAs containing RRE from the nucleus to the cytoplasm and increases the half-life of these viral mRNAs. A third factor, Nef, is a cytoplasmic myristylated protein that has been proposed to down-regulate virus expression. These factors are integrated in a feedback regulatory network that dictates the balanced expression of viral components. The study of HIV expression in human cells will advance our understanding of the mechanisms related to the pathogenicity of HIV. This knowledge may also lead to novel diagnostic and therapeutic approaches to combat AIDS.

L13 ANSWER 4 OF 20 MEDLINE
ACCESSION NUMBER: 80094875 MEDLINE
DOCUMENT NUMBER: 80094875 PubMed ID: 521529
TITLE: Ultrastructural and cell surface changes of human psoriatic skin following Goeckerman therapy.
AUTHOR: Lupulescu A P; Chadwick J M; Downham T F 2nd
SOURCE: JOURNAL OF CUTANEOUS PATHOLOGY, (1979 Oct) 6 (5) 347-63.
Journal code: 0425124. ISSN: 0303-6987.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198003
ENTRY DATE: Entered STN: 19900315

Entered Medline: 19800317

AB Ultrastructural and cell surface studies of skin in psoriatic patients prior to and after Goeckerman therapy (crude coal ***tar*** and UVB-light) have demonstrated significant cellular changes following this treatment: hyperactivity of melanocytes with melanosome polymorphism, increase of desmosomes, tonofilaments, keratohyaline granules, a decrease in mitochondria, keratinosomes, polysomes, dark cells and a reduction in size of nuclei and ***nucleoli*** . The enlargement of intercellular spaces and the redundancy of basement membrane were also reduced. Langerhans cells were moderately decreased and exhibited a normal ultrastructural pattern. No significant changes in cutaneous nerve distribution or morphology were observed in these cases. Scanning electron microscopy following treatment revealed a regular surface and orientation of corneocytes, with flattened surfaces; and a reduction of their ridges on the surfaces, as well as of the intercellular spaces and red blood cells. These findings indicate that Goeckerman therapy restored the ultrastructural and cell surface pattern in the psoriatic skin by inducing orthokeratinogenesis, development of the tonofibrillar-desmosome system, and decrease in mitochondria, nuclei and ***nucleoli*** .

L13 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1984:485369 CAPLUS
DOCUMENT NUMBER: 101:85369
TITLE: The effects of ***tar*** dyes on RNA synthesis. Part 5. Configurational changes in rat liver nuclear chromatin caused by azo dyes
AUTHOR(S): Yoshimoto, M.; Yamaguchi, M.; Hatano, S.; Watanabe, T.
CORPORATE SOURCE: Fac. Agric., Kyushu Univ., Fukuoka, 812, Japan
SOURCE: Food and Chemical Toxicology (1984), 22(5), 3374-44
CODEN: FCTOD7; ISSN: 0278-6915
DOCUMENT TYPE: Journal
LANGUAGE: English
GI

/ Structure 1 in file .gra /

AB The effects of azo dyes on the chromatin configuration of isolated rat liver nuclei were examd. by electron microscopy for elucidation of the mechanism of dye-stimulated in vitro RNA synthesis. Sunset Yellow FCF [2783-94-0] had no effect on the incorporation of [3H]UTP into RNA but amaranth (I) [915-67-3] and Ponceau 3R [3564-09-8] stimulated incorporation .apprx.2- and 3.5-fold, resp. Sunset Yellow FCF did not produce any major alteration in liver nuclei. Isolated liver nuclei treated with I or Ponceau 3R showed dissocn. of heterochromatin and the ***nucleoli*** lost their fine-granular structure acquiring a coarse-granular configuration. The dense structure of heterochromatin in liver nuclei treated with Ponceau 3R was completely dissocd. and appeared as a lace-like configuration. Thus, both Ponceau 3R and I stimulate in vitro RNA synthesis by causing the dissocn. of heterochromatin in isolated rat liver nuclei.

L13 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1964:495149 CAPLUS
DOCUMENT NUMBER: 61:95149
ORIGINAL REFERENCE NO.: 61:16588c-e
TITLE: Content and state of nucleic acids in tissues undergoing inflammatory processes
AUTHOR(S): Varfolomeeva, Z. N.
SOURCE: Biol. Nukleinovogo Obmena u Rast., Akad. Nauk SSSR, Bashkirsk. Filial, Materialy 2-oi Nauchn. [Vtoroi] Konf., Ufa (1964), 1962, 111-14
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB A rise in nucleic acids is known to occur in recovering wound tissue of both plants and animals. In regenerating cells, labile ribonucleic acid predominates in the cytoplasm; in differentiated tissue, the stable form. Labile deoxyribonucleic acid (DNA) in the nucleus stains red in the pyronine-methyl green test; the stable, green. In rabbit pancreas subjected to a coal ***tar*** irritant, cells in direct contact necrosed, with disappearing nucleic acids, as did older ones at some distance, but younger ones passed through 3 stages of change: (1) regression or dedifferentiation, (2) proliferation, and (3) redifferentiation. In 1 the nucleic acids fell sharply in the epithelial cells. In 2 they rose above normal; the inflammatory zone was

nucleoli (red). In 3 a gradual return to normal occurred, with the nucleus still staining green and ***nucleoli*** absent.

L13 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1956:21176 CAPLUS
DOCUMENT NUMBER: 50:21176
ORIGINAL REFERENCE NO.: 50:4373b-d
TITLE: Early cytologic changes produced by carcinogens
AUTHOR(S): Cooper, Norman S.
CORPORATE SOURCE: New York Univ.-Belluvue Med. Center, New York, NY
SOURCE: Bull. N.Y. Acad. Med. (1956), 32, 79-80
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB Enlargement of mouse epidermal cell ***nucleoli*** was used as a criterion of the carcinogenicity of a substance painted on the skin of the animal. U.S.P. white mineral oil caused enlargement, therefore only materials giving enlargements greater than this were considered pos. Cigaret-smoke condensate (50% in acetone) was pos.; 50% coal ***tar*** in acetone gave even greater enlargement. The greatest enlargement was given by 0.5% 3,4-benzopyrene in acetone. A 50% soln. of turpentine in acetone produced nucleolar enlargement only slightly less than that resulting from tobacco ***tar*** .

L13 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1928:18650 CAPLUS
DOCUMENT NUMBER: 22:18650
ORIGINAL REFERENCE NO.: 22:2199b-e
TITLE: Studies in the microchemistry of the cell. I. The chromatin content of normal and malignant cells as demonstrated by Feulgen's "Nuclealreaktion"
AUTHOR(S): Ludford, R. J.
SOURCE: Proc. Roy. Soc. (London) (1928), B102, 397-406
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB The following results were obtained by use of this reaction. In the rat and the mouse, the chromatin content of the nucleus does not increase during oogenesis, and no chromatin is extruded into the cytoplasm when the germinal vesicle breaks down to form the chromosomes. Chromatin is not present in either the oxyphilic or the basophilic ***nucleoli*** of the oocyte of the mollusk, Limnoea stagnalis. The sperm heads of all 3 species yield a faint reaction when the chromosomes are stretched; but the reaction becomes progressively more marked as condensation occurs in spermatogenesis. The chromosomes apparently contain other substances besides chromatin. Extrusion of chromatin does not occur in the epithelial cells of the epididymis. Shrinkage of the nuclei of gland cells after secretion (e. g., in the adrenal medulla after exposure to cold) is apparently due to the loss of some substance other than chromatin. No relationship is found between the chromatin content of a tumor cell nucleus and the rate of growth of the tumor. In a ***tar*** tumor and the surrounding skin, the chromatin content is apparently the same in the normal and the malignant cells. During cellular degeneration of tumors, their nuclei become shrunken and the chromatin runs together. In tumors, the chromosomes stain intensely, and are separate from the nucleolus during the prophase of nitosis. Nuclear extrusions, which are well marked in some tumors, consist of nucleolar material and not chromatin. Nucleolar extrusions are the chief source of keratohyalin during cornification. While the same amt. of chromatin may be present in both large and small nuclei, yet giant nuclei contain large masses of chromatin.

L13 ANSWER 9 OF 20 USPATFULL
ACCESSION NUMBER: 2002:279998 USPATFULL
TITLE: Genetically engineered herpes virus for the treatment of cardiovascular disease
INVENTOR(S): Schwartz, Lewis B., Hinsdale, IL, UNITED STATES
Weichselbaum, Ralph R., Chicago, IL, UNITED STATES
Roizman, Bernard, Chicago, IL, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002155432	A1	20021024	
APPLICATION INFO.:	US 2001-995475	A1	20011128	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-253680P	20001128 (60)

FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MARSHALL, GERSTEIN & BORUN, 6300 SEARS TOWER, 233 SOUTH WACKER, CHICAGO, IL, 60606-6357
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
LINE COUNT: 4203
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods of expressing a nucleic acid or producing a proteinaceous composition encoded by a nucleic acid in vascular and cardiovascular cells by administration of a herpesvirus vector. The present invention provides methods of producing a therapeutic benefit in vascular and cardiovascular tissue by administration of a herpesvirus vector. In additional aspects, the invention concerns combination therapies for vascular and cardiovascular diseases comprising administration of a herpesvirus vector and treatment with at least one addition pharmacological agent or surgical procedure.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 10 OF 20 USPATFULL
ACCESSION NUMBER: 2002:217408 USPATFULL
TITLE: Oligomers that bind to ku protein
INVENTOR(S): Dynan, William S., Augusta, GA, United States
Yoo, Sunghan, Augusta, GA, United States
PATENT ASSIGNEE(S): Medical College of Georgia Research Institute, Inc., Augusta, GA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6441158	B1	20020827
APPLICATION INFO.:	US 1998-223139		19981230 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-70278P	19971231 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Wang, Andrew
LEGAL REPRESENTATIVE: Kilpatrick Stockton LLP, Rothschild, Cynthia B., Calkins, Charles W.
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 2333
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are oligomers that bind Ku protein. These oligomers are useful for inhibiting activation of DNA-PK, treating certain forms of autoimmune disease, detection and purification of Ku protein, and identification of proteins that interact with Ku protein. Preferably, the oligomers are composed of nucleotides, nucleotide analogs, or a combination. Most preferably, the oligomers are composed of ribonucleotides. Also disclosed is a method of inhibiting DNA repair, a method of identifying cellular proteins that interact with Ku protein, and a method of treating autoimmune disease in patients with anti-Ku antibodies. The disclosed oligomers can have several preferred features, either alone or in combination, in addition to Ku binding. One such feature, referred to herein as inhibition activity, is inhibition of DNA-PK kinase activity. Another preferred feature, referred to herein as aptamer motifs, is the presence of one or more of the base sequences GCUUUCCCANNNAC, A(A/C)AUGA, and AACUUCGA. These sequences--referred to herein as aptamer motif 1, aptamer motif 2, and aptamer motif 3, respectively--are associated with Ku binding capability. Another preferred feature, referred to herein as aptamer structure, is the presence of a structure similar to the structure shown in FIG. 6A. This structure has the general formula 5'-A-B-C-D-C'-E-A'-3', where A, B, C, D, C', E, and A' are components of the oligomer. In this structure, A and A' interact to form a stem structure, C and C' interact to form a stem structure, B and E make up a bulge region, and D is either a bulge or a loop. FIG. 6A depicts component D as a loop. Each of these preferred features (inhibition activity, aptamer motif, and aptamer structure) can be used either alone or in combination with one or both of the other characteristics.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 11 OF 20 USPATFULL

TITLE: . HIV chemokines
INVENTOR(S): Ludwig, Linda B., East Aurora, NY, United States
Ambrus, Jr., Julian L., Buffalo, NY, United States
Krawczyk, Kristie Anne, Gowanda, NY, United States
PATENT ASSIGNEE(S): The Research Foundation of State University of New York, Amherst, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6392029	B1	20020521
APPLICATION INFO.:	US 1999-249542		19990212 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1997-853703, filed on 9 May 1997, now patented, Pat. No. US 5919677

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-74640P	19980213 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Bui, Phuong T.
LEGAL REPRESENTATIVE: Hodgson Russ LLP
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 19 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 1868
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a gene comprising an open reading frame encoded on the plus strand of the pro-viral DNA, and located in the region of HIV-1 long terminal repeat. The gene encodes a protein that is related to, and has a structural motif resembling that of chemokine proteins. Depending upon the ribosomal frameshift, a plurality of proteins may be translated from the antisense RNA. The protein has similarity with chemokine SDF-1 and may play a role as a cofactor with gp120 in the binding to and entry of HIV to a target cell.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 12 OF 20 USPATFULL
ACCESSION NUMBER: 2001:202200 USPATFULL
TITLE: Tat-derived transport polypeptides
INVENTOR(S): Frankel, Alan, Tiburon, CA, United States
Pabo, Carl, Newton, MA, United States
Barsoum, James G., Lexington, MA, United States
Fawell, Stephen E., Winchester, MA, United States
Pepinsky, R. Blake, Arlington, MA, United States
PATENT ASSIGNEE(S): Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)
Johns Hopkins Univ. School of Medicine, Baltimore, MD, United States (U.S. corporation)
Biogen, Inc., Cambridge, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6316003	B1	20011113
APPLICATION INFO.:	US 1994-235403		19940428 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-158015, filed on 24 Nov 1993, now abandoned Continuation of Ser. No. US 1991-636662, filed on 2 Jan 1991, now abandoned Continuation-in-part of Ser. No. US 1989-454450, filed on 21 Dec 1989, now abandoned , said Ser. No. US 636662 And Ser. No. WO 1993-US7833, filed on 19 Aug 1993 , said Ser. No. US 636662 And Ser. No. US 1992-934375, filed on 21 Aug 1992, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: MacMillan, Keith D.
ASSISTANT EXAMINER: Wessendorf, T. D.
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 21 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 3392
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to delivery of biologically active cargo molecules, such as polypeptides and nucleic acids, into the cytoplasm and nuclei of cells in vitro and in vivo. Intracellular delivery of

of novel transport polypeptides which include HIV tat protein or one or more portions thereof, and which are covalently attached to cargo molecules. The transport polypeptides in preferred embodiments of this invention are characterized by the presence of the tat basic region (amino acids 49-57), the absence of the tat cysteine-rich region (amino acids 22-36) and the absence of the tat exon 2-encoded carboxy-terminal domain (amino acids 73-86) of the naturally-occurring tat protein. By virtue of the absence of the cysteine-rich region, the preferred transport polypeptides of this invention solve the potential problems of spurious trans-activation and disulfide aggregation. The reduced size of the preferred transport polypeptides of this invention also minimizes interference with the biological activity of the cargo molecule.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 13 OF 20 USPATFULL
ACCESSION NUMBER: 1998:108434 USPATFULL
TITLE: Tat-derived transport polypeptides and fusion proteins
INVENTOR(S): Frankel, Alan, Tiburon, CA, United States
Pabo, Carl, Newton, MA, United States
Barsoum, James G., Lexington, MA, United States
Fawell, Stephen E., Winchester, MA, United States
Pepinsky, R. Blake, Arlington, MA, United States
PATENT ASSIGNEE(S): Biogen, Inc., Cambridge, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5804604		19980908
APPLICATION INFO.:	US 1995-450236		19950525 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-235403, filed on 28 Apr 1994 which is a continuation-in-part of Ser. No. US 1993-158015, filed on 24 Nov 1993, now abandoned which is a continuation of Ser. No. US 1991-636662, filed on 2 Jan 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-454450, filed on 21 Dec 1989, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Elliott, George C.
ASSISTANT EXAMINER: Larson, Thomas G.
LEGAL REPRESENTATIVE: Biogen, Inc., Kaplan, Warren A.
NUMBER OF CLAIMS: 14
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 26 Drawing Figure(s); 21 Drawing Page(s)
LINE COUNT: 4456
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to delivery of biologically active cargo molecules, such as polypeptides and nucleic acids, into the cytoplasm and nuclei of cells in vitro and in vivo. Intracellular delivery of cargo molecules according to this invention is accomplished by the use of novel transport polypeptides which comprise HIV tat protein or one or more portions thereof, and which are covalently attached to cargo molecules. The transport polypeptides in preferred embodiments of this invention are characterized by the presence of the tat basic region (amino acids 49-57), the absence of the tat cysteine-rich region (amino acids 22-36) and the absence of the tat exon 2-encoded carboxy-terminal domain (amino acids 73-86) of the naturally-occurring tat protein. By virtue of the absence of the cysteine-rich region, the preferred transport polypeptides of this invention solve the potential problems of spurious trans-activation and disulfide aggregation. The reduced size of the preferred transport polypeptides of this invention also minimizes interference with the biological activity of the cargo molecule.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 14 OF 20 USPATFULL
ACCESSION NUMBER: 1998:48556 USPATFULL
TITLE: Tat-derived transport polypeptide conjugates
INVENTOR(S): Frankel, Alan, 21 Marinero Cir., #206, Tiburon, CA, United States 94920
Pabo, Carl, 18 Weldon Rd., Newton, MA, United States 02158
Barsoum, James G., 9 Marlboro Rd., Lexington, MA, United States 02173
Fawell, Stephen E., One Black Horse Ter., Winchester,

Pepinsky, R. Blake, 30 Falmouth Rd., Arlington, MA, United States 02174

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5747641		19980505
APPLICATION INFO.:	US 1995-451233		19950525 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-235403, filed on 28 Apr 1994 which is a continuation-in-part of Ser. No. US 1993-158015, filed on 24 Nov 1993, now abandoned which is a continuation of Ser. No. US 1991-636662, filed on 2 Jan 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-454450, filed on 21 Dec 1989, now abandoned And a continuation-in-part of Ser. No. US -235403 which is a continuation-in-part of Ser. No. US 1992-934375, filed on 12 Aug 1992, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Elliott, George C.
ASSISTANT EXAMINER: Larson, Thomas G.
LEGAL REPRESENTATIVE: Fish & Neave, Haley, Jr., James F., Kanter, Madge R.
NUMBER OF CLAIMS: 5
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 21 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 3850
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to delivery of biologically active cargo molecules, such as polypeptides and nucleic acids, into the cytoplasm and nuclei of cells in vitro and in vivo. Intracellular delivery of cargo molecules according to this invention is accomplished by the use of novel transport polypeptides which comprise HIV tat protein or one or more portions thereof, and which are covalently attached to cargo molecules. The transport polypeptides in preferred embodiments of this invention are characterized by the presence of the tat basic region (amino acids 49-57), the absence of the tat cysteine-rich region (amino acids 22-36) and the absence of the tat exon 2-encoded carboxy-terminal domain (amino acids 73-86) of the naturally-occurring tat protein. By virtue of the absence of the cysteine-rich region, the preferred transport polypeptides of this invention solve the potential problems of spurious trans-activation and disulfide aggregation. The reduced size of the preferred transport polypeptides of this invention also minimizes interference with the biological activity of the cargo molecule.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 15 OF 20 USPATFULL
ACCESSION NUMBER: 97:106804 USPATFULL
TITLE: Cellular protein TDP-43 and regulation of HIV-1 gene expression
INVENTOR(S): Gaynor, Richard B., Dallas, TX, United States
Ou, S.-H. Iqnatius, Dallas, TX, United States
Wu, Foon Kin, Carrollton, TX, United States
PATENT ASSIGNEE(S): Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5688511		19971118
APPLICATION INFO.:	US 1994-343682		19941122 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1991-788266, filed on 5 Nov 1991, now patented, Pat. No. US 5350835 And Ser. No. US 1994-239047, filed on 6 May 1994, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Nucker, Christine M.
ASSISTANT EXAMINER: Stucker, Jeffrey
LEGAL REPRESENTATIVE: Arnold, White & Durkee
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 2863
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions including a polypeptide or nucleic acid sequence encoding a polypeptide that binds ***TAR*** DNA (particularly the region -18 to

(particularly the region +1 to +80 of the ***TAR*** RNA) are disclosed. The cellular binding protein TDP-43 including the polypeptide has an estimated molecular weight of between about 40 kD and 46 kD as determined by SDS polyacrylamide gel electrophoresis. Fusion proteins that include the entire cellular binding protein TDP-43 or fragments thereof are also described. The cellular binding protein, peptide fragments and nucleic acid sequences encoding them, repress HIV gene expression. Methods for preparing the cellular binding protein from cells as recombinant proteins with recombinant host cells are also disclosed. Antibodies to the TDP-43 cellular binding protein are also described. The isolated nucleic acid sequences of the protein and its fragments are described in the construction of retroviral vectors. Methods for using the cellular binding protein, polypeptides thereof, and nucleic acid sequences encoding the protein and its fragments in the preparation of pharmaceutical preparations and in methods for repressing HIV gene expression are also disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 16 OF 20 USPATFULL

ACCESSION NUMBER: 97:91635 USPATFULL
TITLE: Fusion protein comprising tat-derived transport moiety
INVENTOR(S): Frankel, Alan, 21 Marinero Cir. #206, Tiburon, CA, United States 94920
Pabo, Carl, 18 Weldon Rd., Newton, MA, United States 02158
Barsoum, James G., 9 Marlboro Rd., Lexington, MA, United States 02173
Fawell, Stephen E., One Black Horse Ter., Winchester, MA, United States 01890
Pepinsky, R. Blake, 30 Falmouth Rd., Arlington, MA, United States 02174

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5674980		19971007
APPLICATION INFO.:	US 1995-450098		19950525 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-235403, filed on 28 Apr 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-158015, filed on 24 Nov 1993, now abandoned which is a continuation of Ser. No. US 1991-636662, filed on 2 Jan 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-454450, filed on 21 Dec 1989, now abandoned , said Ser. No. US -235403 which is a continuation-in-part of Ser. No. US 1992-934375, filed on 21 Aug 1992, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Elliott, George C.
ASSISTANT EXAMINER: Larson, Thomas G.
LEGAL REPRESENTATIVE: Fish & Neave, Haley, Jr., James F., Kanter, Madge R.
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 21 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 3855
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to delivery of biologically active cargo molecules, such as polypeptides and nucleic acids, into the cytoplasm and nuclei of cells in vitro and in vivo. Intracellular delivery of cargo molecules according to this invention is accomplished by the use of novel transport polypeptides which comprise HIV tat protein or one or more portions thereof, and which are covalently attached to cargo molecules. The transport polypeptides in preferred embodiments of this invention are characterized by the presence of the tat basic region (amino acids 49-57), the absence of the tat cysteine-rich region (amino acids 22-36) and the absence of the tat exon 2-encoded carboxy-terminal domain (amino acids 73-86) of the naturally-occurring tat protein. By virtue of the absence of the cysteine-rich region, the preferred transport polypeptides of this invention solve the potential problems of spurious trans-activation and disulfide aggregation. The reduced size of the preferred transport polypeptides of this invention also minimizes interference with the biological activity of the cargo molecule.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 17 OF 20 USPATFULL

TITLE: Nucleic acid conjugates of tat-derived transport polypeptides

INVENTOR(S): Frankel, Alan, 21 Marinero Cir. #206, Tiburon, CA, United States 94920
Pabo, Carl, 18 Weldon Rd., Newton, MA, United States 02158
Barsoum, James G., 9 Marlboro Rd., Lexington, MA, United States 02173
Fawell, Stephen E., One Black Horse Ter., Winchester, MA, United States 01890
Pepinsky, R. Blake, 30 Falmouth Rd., Arlington, MA, United States 02174

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5670617		19970923
APPLICATION INFO.:	US 1995-450246		19950525 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1994-235403, filed on 28 Apr 1994 which is a continuation-in-part of Ser. No. US 1993-158015, filed on 24 Nov 1993, now abandoned which is a continuation of Ser. No. US 1991-636662, filed on 2 Jan 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-454450, filed on 21 Dec 1989, now abandoned , said Ser. No. US -235403 which is a continuation-in-part of Ser. No. US 1992-934375, filed on 21 Aug 1992, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Elliot, George C.
ASSISTANT EXAMINER: Larson, Thomas G.
LEGAL REPRESENTATIVE: Fish & Neave, Haley, Jr., James F., Kanter, Madge R.
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 21 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 3849
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to delivery of biologically active cargo molecules, such as polypeptides and nucleic acids, into the cytoplasm and nuclei of cells in vitro and in vivo. Intracellular delivery of cargo molecules according to this invention is accomplished by the use of novel transport polypeptides which comprise HIV tat protein or one or more portions thereof, and which are covalently attached to cargo molecules. The transport polypeptides in preferred embodiments of this invention are characterized by the presence of the tat basic region (amino acids 49-57), the absence of the tat cysteine-rich region (amino acids 22-36) and the absence of the tat exon 2-encoded carboxy-terminal domain (amino acids 73-86) of the naturally-occurring tat protein. By virtue of the absence of the cysteine-rich region, the preferred transport polypeptides of this invention solve the potential problems of spurious trans-activation and disulfide aggregation. The reduced size of the preferred transport polypeptides of this invention also minimizes interference with the biological activity of the cargo molecule.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 18 OF 20 USPATFULL

ACCESSION NUMBER: 97:66013 USPATFULL

TITLE: Nucleic acids encoding and methods of making tat-derived transport polypeptides

INVENTOR(S): Frankel, Alan, 21 Marinero Cir., Tiburon, CA, United States 94920
Pabo, Carl, 18 Weldon Rd., Newton, MA, United States 02158
Barsoum, James G., 9 Marlboro Rd., Lexington, MA, United States 02173
Fawell, Stephen E., One Black Horse Ter., Winchester, MA, United States 01890
Pepinsky, R. Blake, 30 Falmouth Rd., Arlington, MA, United States 02174

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5652122		19970729
APPLICATION INFO.:	US 1995-450257		19950525 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1994-235403, filed on 28 Apr 1994 which is a continuation-in-part of Ser. No. US

is a continuation of Ser. No. US 1991-636662, filed on 2 Jan 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-454450, filed on 21 Dec 1989, now abandoned , said Ser. No. US -235403 which is a continuation-in-part of Ser. No. US 1992-934375, filed on 21 Aug 1992, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Elliott, George C.
ASSISTANT EXAMINER: Larson, Thomas G.
LEGAL REPRESENTATIVE: Fish & Neave, Haley, Jr., James F., Kanter, Madge R.
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 21 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 3872
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to delivery of biologically active cargo molecules, such as polypeptides and nucleic acids, into the cytoplasm and nuclei of cells in vitro and in vivo. Intracellular delivery of cargo molecules according to this invention is accomplished by the use of novel transport polypeptides which comprise HIV tat protein or one or more portions thereof, and which are covalently attached to cargo molecules. The transport polypeptides in preferred embodiments of this invention are characterized by the presence of the tat basic region (amino acids 49-57), the absence of the tat cysteine-rich region (amino acids 22-36) and the absence of the tat exon 2-encoded carboxy-terminal domain (amino acids 73-86) of the naturally-occurring tat protein. By virtue of the absence of the cysteine-rich region, the preferred transport polypeptides of this invention solve the potential problems of spurious trans-activation and disulfide aggregation. The reduced size of the preferred transport polypeptides of this invention also minimizes interference with the biological activity of the cargo molecule.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 19 OF 20 USPATFULL
ACCESSION NUMBER: 96:1197 USPATFULL
TITLE: Apparatus kit for detecting Dientamoeba fragilis
INVENTOR(S): Riordan, Neil H., 7715 E. 32nd N., Wichita, KS, United States 67226

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5480613		19960102
APPLICATION INFO.:	US 1994-239731		19940509 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1992-964874, filed on 22 Oct 1992, now patented, Pat. No. US 5334509, issued on 2 Aug 1994
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Snay, Jeffrey R.
ASSISTANT EXAMINER: Wallenhorst, Maureen M.
LEGAL REPRESENTATIVE: Hovey, Williams, Timmons & Collins
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 14 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 612
AB A method and apparatus for producing detectable intestinal parasites. The method includes obtaining an intestinal mucosa sample (e.g. feces) having intestinal parasites, such as Dientamoeba fragilis; and contacting the obtained intestinal mucosa sample with an acridine base compound (e.g. acridine orange and/or acridine yellow, etc.) such that the intestinal parasites become differentially stained and detectable by a human eye when viewed through a fluorescence microscope. The apparatus includes a kit or the like which includes at least one vessel or vial. Preferably, two vials are contained within the kit with one vial having an isotonic salt solution including a salt, sodium chloride, potassium phosphate, etc., and the other vial containing an acridine biological staining compound.

L13 ANSWER 20 OF 20 USPATFULL
ACCESSION NUMBER: 94:66402 USPATFULL
TITLE: Method for detecting intestinal pathogen dientamoeba fragilis
INVENTOR(S): Riordan, Neil H., 7715 E. 32nd North, Wichita, KS,

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5334509		19940802	
APPLICATION INFO.:	US 1992-964874		19921022	(7)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Housel, James C.
ASSISTANT EXAMINER: Wallenhorst, Maureen M.
LEGAL REPRESENTATIVE: Carpenter, John W.
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 15
NUMBER OF DRAWINGS: 14 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 681

AB A method and apparatus for producing detectable intestinal parasites. The method includes obtaining an intestinal mucosa sample (e.g. feces) having intestinal parasites, such as Dienamoeba fragilis; and contacting the obtained intestinal mucosa sample with an acridine base compound (e.g. acridine orange and/or acridine yellow, etc.) such that the intestinal parasites become differentially stained and detectable by a human eye when viewed through a fluorescence microscope. The apparatus includes a kit or the like which includes at least one vessel or vial. Preferably, two vials are contained within the kit with one vial having an isotonic salt solution including a salt, such as sodium chloride, potassium phosphate, etc., and the other vial containing an acridine biological staining compound.

=> s tar and hiv and rna
L14 3170 TAR AND HIV AND RNA

=> s l14 and nucle?
L15 1920 L14 AND NUCLE?

=> s l14 and snorna
L16 1 L14 AND SNORNA

=> d l16 ibib abs tot

L16 ANSWER 1 OF 1 USPATFULL
ACCESSION NUMBER: 1999:81758 USPATFULL
TITLE: Non-activated receptor complex proteins and uses thereof
INVENTOR(S): Davis, Roger J., Princeton, MA, United States
Galcheva-Gargova, Zoya, Worcester, MA, United States
PATENT ASSIGNEE(S): University of Massachusetts, Boston, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5925566		19990720	
APPLICATION INFO.:	US 1997-870518		19970606	(8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-19219P	19960606 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Campell, Bruce R.
ASSISTANT EXAMINER: Nguyen, Dave Trong
LEGAL REPRESENTATIVE: Fish & Richardson, P.C.
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 22 Drawing Figure(s); 18 Drawing Page(s)
LINE COUNT: 2438
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention features a substantially pure ZPR1 polypeptide. For example, a ZPR1 polypeptide that specifically binds to a non-activated membrane-bound receptor (e.g., EGF or PDGF receptors) and specifically binds small nucleolar RNAs (e.g., U3). ZPR1 polypeptides can be isolated from any eukaryote, including mammals (e.g. rodents and humans) and fungi (e.g., S. cerevisiae and S. pombe).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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=> s l14 and (small nucleolar)
L17          7 L14 AND (SMALL NUCLEOLAR)

=> s l17 not l16
L18          6 L17 NOT L16

=> d l18 ibib abs tot

L18  ANSWER 1 OF 6     MEDLINE
ACCESSION NUMBER:    2002647867     MEDLINE
DOCUMENT NUMBER:     22294961   PubMed ID: 12376617
TITLE:               A nucleolar   ***TAR***   decoy inhibitor of   ***HIV***
                     -1 replication.
AUTHOR:              Michienzi Alessandro; Li Shirley; Zaia John A; Rossi John J
CORPORATE SOURCE:    Divisions of Molecular Biology and Virology, Beckman
                     Research Institute of the City of Hope, 1450 East Duarte
                     Road, Duarte, CA 91010-3011, USA.
CONTRACT NUMBER:     AI29329 (NIAID)
     AI46030 (NIAID)
SOURCE:              PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE
                     UNITED STATES OF AMERICA, (2002 Oct 29) 99 (22) 14047-52.
                     Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200212
ENTRY DATE:          Entered STN: 20021031
                     Last Updated on STN: 20021217
                     Entered Medline: 20021209
AB   Tat is a critical regulatory factor in   ***HIV***  -1 gene expression. It
     mediates the transactivation of transcription from the   ***HIV***  -1 LTR
     by binding to the transactivation response (  ***TAR***  ) element in a
     complex with cyclin T1. Because of its critical and early role in
       ***HIV***   gene expression, Tat and its interaction with the   ***TAR***
     element constitute important therapeutic targets for the treatment of
       ***HIV***  -1 infection. Based on the known nucleolar localization
     properties of Tat, we constructed a chimeric   ***small***
       ***nucleolar***     ***RNA***  -  ***TAR***   decoy that localizes to the
     nucleoli of human cells and colocalizes in the nucleolus with a
     Tat-enhanced GFP fusion protein. When the chimeric   ***RNA***   was
     stably expressed in human T lymphoblastoid CEM cells it potently inhibited
       ***HIV***  -1 replication. These results demonstrate that the nucleolar
     trafficking of Tat is critical for   ***HIV***  -1 replication and
     suggests a role for the nucleolus in   ***HIV***  -1 viral replication.

L18  ANSWER 2 OF 6  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:        2002:859140  CAPLUS
DOCUMENT NUMBER:         137:336616
TITLE:                   A nucleolar   ***TAR***   decoy inhibitor of
                           ***HIV***  -1 replication
AUTHOR(S):               Michienzi, Alessandro; Li, Shirley; Zaia, John A.;
                         Rossi, John J.
CORPORATE SOURCE:        Division of Molecular Biology, Beckman Research
                         Institute of the City of Hope, Duarte, CA, 91010-3011,
                         USA
SOURCE:                  Proceedings of the National Academy of Sciences of the
                         United States of America (2002), 99(22), 14047-14052
                         CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER:               National Academy of Sciences
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   Tat is a crit. regulatory factor in   ***HIV***  -1 gene expression.  It
     mediates the transactivation of transcription from the   ***HIV***  -1 LTR
     by binding to the transactivation response (  ***TAR***  ) element in a
     complex with cyclin T1.  Because of its crit. and early role in
       ***HIV***   gene expression, Tat and its interaction with the   ***TAR***
     element constitute important therapeutic targets for the treatment of
       ***HIV***  -1 infection.  Based on the known nucleolar localization
     properties of Tat, the authors constructed a chimeric   ***small***
       ***nucleolar***     ***RNA***  -  ***TAR***   decoy that localizes to the
     nucleoli of human cells and colocalizes in the nucleolus with a
     Tat-enhanced GFP fusion protein.  When the chimeric   ***RNA***   was
     stably expressed in human T lymphoblastoid CEM cells it potently inhibited
       ***HIV***  -1 replication.  These results demonstrate that the nucleolar
     trafficking of Tat is crit. for   ***HIV***  -1 replication and suggests a
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REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 3 OF 6 LIFESCI COPYRIGHT 2002 CSA
ACCESSION NUMBER: 2002:119753 LIFESCI
TITLE: A nucleolar ***TAR*** decoy inhibitor of ***HIV*** -1 replication
AUTHOR: Michienzi, A.; Li, S.; Zaia, J.A.; Rossi, J.J.
CORPORATE SOURCE: Divisions of Molecular Biology and Virology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010-3011; E-mail: Jrossi@bricoh.edu
SOURCE: Proceedings of the National Academy of Sciences, USA [Proc. Natl. Acad. Sci. USA], (20021029) vol. 99, no. 22, pp. 14047-14052.
ISSN: 0027-8424.
DOCUMENT TYPE: Journal
FILE SEGMENT: N; V
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Tat is a critical regulatory factor in ***HIV*** -1 gene expression. It mediates the transactivation of transcription from the ***HIV*** -1 LTR by binding to the transactivation response (***TAR***) element in a complex with cyclin T1. Because of its critical and early role in ***HIV*** gene expression, Tat and its interaction with the ***TAR*** element constitute important therapeutic targets for the treatment of ***HIV*** -1 infection. Based on the known nucleolar localization properties of Tat, we constructed a chimeric ***small*** ***nucleolar*** ***RNA*** - ***TAR*** decoy that localizes to the nucleoli of human cells and colocalizes in the nucleolus with a Tat-enhanced GFP fusion protein. When the chimeric ***RNA*** was stably expressed in human T lymphoblastoid CEM cells it potently inhibited ***HIV*** -1 replication. These results demonstrate that the nucleolar trafficking of Tat is critical for ***HIV*** -1 replication and suggests a role for the nucleolus in ***HIV*** -1 viral replication.

L18 ANSWER 4 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2002394185 EMBASE
TITLE: A nucleolar ***TAR*** decoy inhibitor of ***HIV*** -1 replication.
AUTHOR: Michienzi A.; Li S.; Zaia J.A.; Rossi J.J.
CORPORATE SOURCE: J.J. Rossi, Divisions of Molecular Biology, Beckman Res. Inst. the City of Hope, 1450 East Duarte Road, Duarte, CA 91010-3011, United States. jrossi@bricoh.edu
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (29 Oct 2002) 99/22 (14047-14052).
Refs: 52
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Tat is a critical regulatory factor in ***HIV*** -1 gene expression. It mediates the transactivation of transcription from the ***HIV*** -1 LTR by binding to the transactivation response (***TAR***) element in a complex with cyclin T1. Because of its critical and early role in ***HIV*** gene expression, Tat and its interaction with the ***TAR*** element constitute important therapeutic targets for the treatment of ***HIV*** -1 infection. Based on the known nucleolar localization properties of Tat, we constructed a chimeric ***small*** ***nucleolar*** ***RNA*** - ***TAR*** decoy that localizes to the nucleoli of human cells and colocalizes in the nucleolus with a Tat-enhanced GFP fusion protein. When the chimeric ***RNA*** was stably expressed in human T lymphoblastoid CEM cells it potently inhibited ***HIV*** -1 replication. These results demonstrate that the nucleolar trafficking of Tat is critical for ***HIV*** -1 replication and suggests a role for the nucleolus in ***HIV*** -1 viral replication.

L18 ANSWER 5 OF 6 USPATFULL
ACCESSION NUMBER: 2002:217408 USPATFULL
TITLE: Oligomers that bind to ku protein
INVENTOR(S): Dynan, William S., Augusta, GA, United States
Yoo, Sunghan, Augusta, GA, United States

Augusta, GA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6441158	B1	20020827
APPLICATION INFO.:	US 1998-223139		19981230 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-70278P	19971231 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Wang, Andrew
LEGAL REPRESENTATIVE: Kilpatrick Stockton LLP, Rothschild, Cynthia B., Calkins, Charles W.
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 2333
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are oligomers that bind Ku protein. These oligomers are useful for inhibiting activation of DNA-PK, treating certain forms of autoimmune disease, detection and purification of Ku protein, and identification of proteins that interact with Ku protein. Preferably, the oligomers are composed of nucleotides, nucleotide analogs, or a combination. Most preferably, the oligomers are composed of ribonucleotides. Also disclosed is a method of inhibiting DNA repair, a method of identifying cellular proteins that interact with Ku protein, and a method of treating autoimmune disease in patients with anti-Ku antibodies. The disclosed oligomers can have several preferred features, either alone or in combination, in addition to Ku binding. One such feature, referred to herein as inhibition activity, is inhibition of DNA-PK kinase activity. Another preferred feature, referred to herein as aptamer motifs, is the presence of one or more of the base sequences GCUUUCCCANNNAC, A(A/C)AUGA, and AACUUCGA. These sequences--referred to herein as aptamer motif 1, aptamer motif 2, and aptamer motif 3, respectively--are associated with Ku binding capability. Another preferred feature, referred to herein as aptamer structure, is the presence of a structure similar to the structure shown in FIG. 6A. This structure has the general formula 5'-A-B-C-D-C'-E-A'-3', where A, B, C, D, C', E, and A' are components of the oligomer. In this structure, A and A' interact to form a stem structure, C and C' interact to form a stem structure, B and E make up a bulge region, and D is either a bulge or a loop. FIG. 6A depicts component D as a loop. Each of these preferred features (inhibition activity, aptamer motif, and aptamer structure) can be used either alone or in combination with one or both of the other characteristics.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L18 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2003:15084 BIOSIS
DOCUMENT NUMBER: PREV200300015084
TITLE: A nucleolar ***TAR*** decoy inhibitor of ***HIV*** -1 replication.
AUTHOR(S): Michienzi, Alessandro; Li, Shirley; Zaia, John A.; Rossi, John J. (1)
CORPORATE SOURCE: (1) Divisions of Molecular Biology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA, 91010-3011, USA: jrossi@bricoh.edu USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (October 29 2002) Vol. 99, No. 22, pp. 14047-14052. print.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Tat is a critical regulatory factor in ***HIV*** -1 gene expression. It mediates the transactivation of transcription from the ***HIV*** -1 LTR by binding to the transactivation response (***TAR***) element in a complex with cyclin T1. Because of its critical and early role in ***HIV*** gene expression, Tat and its interaction with the ***TAR*** element constitute important therapeutic targets for the treatment of ***HIV*** -1 infection. Based on the known nucleolar localization properties of Tat, we constructed a chimeric ***small*** ***nucleolar*** ***RNA*** - ***TAR*** decoy that localizes to the nucleoli of human cells and colocalizes in the nucleolus with a

- stably expressed in human T lymphoblastoid CEM cells it potently inhibited ***HIV*** -1 replication. These results demonstrate that the nucleolar trafficking of Tat is critical for ***HIV*** -1 replication and suggests a role for the nucleolus in ***HIV*** -1 viral replication.

=> d 5 kwic

L18 ANSWER 5 OF 6 USPATFULL
SUMM There is some evidence that Ku protein interacts with ***RNA*** , although this has been much less studied than the interaction with DNA. Antibodies to Ku protein stain both the nucleoplasm. . . not (Reeves, J. Exp. Med. 161, 18-39 (1985)). Thus, nucleolar localization may be regulated by interaction of Ku protein with ***RNA*** . Ku protein does not appear to bind to bulk tRNA or to synthetic ***RNA*** polymers (Mimori & Hardin, J. Biol. Chem. 261, 10375-10379 (1986)). However, one study showed that Ku protein forms a specific complex with an ***RNA*** that included the ***HIV*** trans-activation response (***TAR***) element sequence (Kaczmarski & Khan, Biochem. Biophys. Res. Commun. 196, 935-942 (1993)).
DRWD FIG. 1 is a graph of percent ***RNA*** or DNA bound versus concentration of Ku (nM) for five representative Ku protein- ***RNA*** and DNA binding curves obtained with an electrophoretic mobility shift assay. The graph shows that pooled ***RNA*** tested after the fourth and sixth round of Systematic Evolution of Ligands by Exponential Enrichment (SELEX; Tuerk & Gold, Science. . . and 5,567,588) had an increased ability to bind to Ku protein. Radiolabeled nucleic acid probes were as follows: .quadrature., nonselected ***RNA*** ; .gradient., ***RNA*** after 4th round of selection; .tangle-solidup., ***RNA*** after 6th round of selection; O, ***HIV*** - ***TAR*** ***RNA*** (included for comparison); .diamond-solid., 21 base pair double-stranded DNA.
DRWD FIG. 2A is a graph of kinase activity (percentage of activity in absence of ***RNA***) versus ***RNA*** concentration (nM) for eight representative aptamers. The aptamers were as follows: X, nonselected ***RNA*** ; O, #2; .quadrature., #7-3; .DELTA., SC4; +, SC5; .diamond-solid., SC8; .tangle-soliddn., #1-2; .circle-solid., SC2.
DRWD FIG. 2B is a graph of DNA binding (percent of Ku-DNA complexes detected in the absence of competitor) versus ***RNA*** concentration (nM) for seven representative aptamers. Purified Ku protein (2 nM) was incubated with radiolabeled 21 base pair double-stranded DNA (1 nM) in the presence of various amounts of nonselected ***RNA*** or aptamer. The aptamers were as follows: X, nonselected ***RNA*** ; O, #2; .quadrature., #7-3; .DELTA., SC4; +, SC5; .diamond-solid., SC8; .tangle-soliddn., #1-2; .circle-solid., SC2.
DRWD FIG. 3 is a bar graph of kinase activity (percentage of activity in the absence of ***RNA***) in the presence of different aptamers, each at three different concentrations. Reactions were performed in crude nuclear extract. Reactions contained. . .
DRWD . . . '3, SEQ ID NOs: 22, 6, and 23) and #2 ('5 to '3, SEQ ID NOs: 22, 5, and 23). ***RNA*** secondary structures were predicted using the methods of Zuker and co-workers (Jaeger et al., 1989; Jaeger et al., 1990) as. . .
DETD . . . described in the examples. Only the portion of the selected oligomers that arose from the variable region of the pool ***RNA*** is shown in Table 1. That is, each of the oligonucleotides listed in Table 1 include the sequence GGGAGGAUAUUUUCUCAGACCGUAA(SEQ ID. . .
DETD
TABLE 1

RNA Sequences and Kd values from the SELEX Procedure
Name DNA-PK
Frequency Aligned sequence Inhibition K.sub.d

CLASS I
SC6 (3) GACUCACGAUGGACCAUACGCCUUCCCACUGGUCUUGUUA (SEQ ID. . . ID NO:17) A 0.8 nM
#42 (1) CAUCCUGGUACUCACUUCGACAUCGUACGUUCAAUCAUAC (SEQ. B) NO:18) B 4.5 nM
OTHER
SC13 (3) ACCUUUUUAGACGAACCUCAAAGUACAUUUAGUUGAAAAC (SEQ ID NO:19) B 0.8 nM

RNA sequences are aligned to show maximum sequence identity. The underlined sequence indicates sequence identity within class.
DETD ***RNA*** is relatively labile and can be degraded by a number of ribonucleases. This degradation can be greatly reduced by the. . . and substitutions at the 2'-prime position of the ribonucleotide and by

RNA . In addition a variety of modifications can be made on the nucleobases themselves which both inhibit degradation and which can. . .

DETD In preferred oligomers, the oligonucleotides are made from ***RNA*** . The examples of Ku aptamers obtained as described in the examples are in the form of ***RNA*** . These RNAs were selected from a pool of about 10.sup.14 different ***RNA*** sequences using the systematic evolution of ligands by exponential enrichment (SELEX) procedure. Most of the selected RNAs bind to Ku. . .

DETD . . . the behavior of molecules with each other. These applications can be adapted to define and display the secondary structure of ***RNA*** and DNA molecules.

DETD . . . be synthesized by solid phase .beta.-cyanoethyl phosphoramidite chemistry (Sinha et al., Nucleic Acids Res. 12:4539-4557 (1984)) on any commercially available DNA/ ***RNA*** synthesizer. A preferred method is the 2'-O-tert-butyldimethylsilyl (TBDMS) protection strategy for the ribonucleotides (Usman et al., J. Am. Chem. Soc.. . . its advantageous properties (McCollum and Andrus Tetrahedron Letters 32:4069-4072 (1991)). Fluorescein can be added to the 5'-end of a substrate ***RNA*** during the synthesis by using commercially available fluorescein phosphoramidites. In general, a desired oligomer can be synthesized using a standard ***RNA*** cycle. Upon completion of the assembly, all base labile protecting groups are removed by an 8 hour treatment at 55.degree.. . .

DETD . . . oligomers can also be produced through enzymatic methods, when the nucleotide subunits are available for enzymatic manipulation. For example, the ***RNA*** molecules can be made through in vitro ***RNA*** polymerase T7 reactions. They can also be made by strains of bacteria or cell lines expressing T7, and then subsequently. . .

DETD . . . viral vectors are Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other ***RNA*** viruses, including these viruses with the ***HIV*** backbone. Also preferred are any viral families which share the properties of these viruses which make them suitable for use. . .

DETD . . . chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an ***RNA*** polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of. . .

DETD . . . a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of ***RNA*** strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for. . .

DETD . . . relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of ***RNA*** polymerase and transcription factors, and may contain upstream elements and response elements.

DETD Expression of the disclosed aptamers in cells also requires sequences to direct expression. It is preferred that an ***RNA*** polymerase III (pol III) promoter be used for expression. Pol III promoters generate transcripts that can be engineered to remain. . . capping signal, and termination sequence. Pol III promoters, and other pol III transcription signals, are present in tRNA genes, 5S ***RNA*** genes, small nuclear ***RNA*** genes, and small cytoplasmic ***RNA*** genes. Preferred pol III promoters for use in aptamer expression vectors are the human small nuclear U6 gene promoter and tRNA gene promoters. The use of U6 gene transcription signals to produce short ***RNA*** molecules in vivo is described by Noonberg et al., Nucleic Acids Res. 22:2830-2836 (1994), and the use of tRNA transcription. . .

DETD . . . Biol. Chem. 268:7868-7873 (1993), and Romero and Blackburn, Cell 67:343-353 (1991). The use of pol III promoters for expression of ***RNA*** molecules is also described in WO 95/23225 by Ribozyme Pharmaceuticals, Inc.

DETD ***RNA*** fusion constructs can be produced which aid in the correct and specific targeting of expressed ***RNA*** molecules. These types of ***RNA*** fusion constructs are discussed in detail in Good et al., "Expression of Small Therapeutic RNAs in Cell Nuclei", Gene Therapy, 4:45-54 (1997). Expression vectors based on human tRNA(met) and U6 snRNA promoters are useful for targeting ***RNA*** expression and to render the resulting ***RNA*** transcripts more resistant to degradation.

DETD . . . coupled to the oligomer. As used herein, a capture tag is any compound that can be associated with a synthesized ***RNA*** molecule and which can be used to separate compounds or complexes having the capture tag from those that do not.. . .

coupled to the 5' end of the synthesized ***RNA*** molecule. Preferred capture tags, described in the context of nucleic acid probes, have been described by Syvanen et al., Nucleic. . .

DETD . . . or bind other groups of antibodies. Thus, one can have an antibody to the capture tag and then this antibody:capture tag: ***RNA*** complex can then be purified by binding to an antibody for the antibody portion of the complex.

DETD . . . molecules of choice. For example, a preferred capture tag of this type is one which contains a sulfer atom. An ***RNA*** molecule which is associated with this capture tag can be purified by retention on a thiolpropyl sepharose column. Extensive washing of the column removes unwanted molecules and reduction with .beta.-mercaptoethanol, for example, allows the desired ***RNA*** molecules to be collected after purification under relatively gentle conditions (See Lorsch and Szostak, 1994 for a reduction to practice. . .

DETD B. Identification Of Cellular ***RNA*** Sequences Predicted That Bind Ku Protein

DETD . . . bind tightly to Ku protein is consistent with suggestions that Ku protein function may be regulated to some extent by ***RNA*** in the normal cell. (Reeves, J. Exp. Med. 161, 18-39 (1985)). One site where such interactions might occur is in. . . Med. 161, 18-39 (1985)). A number of studies have shown that DNA-PK and Ku protein have an ability to regulate ***RNA*** polymerase I, which is localized in the nucleolus (Hoff et al., Proc. Natl. Acad. Sci. USA 91, 762-766 (1994); Kuhn. . . however, Ku protein associates with nucleolar RNAs, this would provide a potential targeting mechanism. Nucleoli contain a multitude of discrete ***small*** ***nucleolar*** RNAs, as well as nascent ribosomal ***RNA*** (reviewed in Smith & Steitz, Cell 89, 669-672 (1997)). The consensus sequences present in the aptamers can be used to predict whether particular nucleolar RNAs or other cellular RNAs are likely to bind to Ku protein. Cellular ***RNA*** sequences that become available in public databases can be searched to determine whether there is identity with the consensus sequences. . .

DETD . . . acid-like properties, such as sequence dependent hybridization, that contain at one or more positions, a modification away from a standard ***RNA*** or DNA nucleotide. A preferred example of an oligonucleotide analogue is peptide nucleic acid.

DETD Preparing the ***RNA*** Pool and Screening for Ku Protein Binding

DETD Because there was little previous information about Ku protein-***RNA*** interactions, the ***RNA*** binding properties of Ku protein were systematically investigated using SELEX (systematic evolution of ligands by exponential enrichment) technology. With this. . .

DETD The ***RNA*** pool was prepared by synthesis of single stranded ***RNA*** from a template comprising a region of conserved sequences and a region of randomized and/or biased sequences.

DETD Synthesis of the ***RNA*** Pool

DETD SELEX protocols and the template used to synthesize the nonselected ***RNA*** pool were obtained from Dr. Hang Chen and Dr. Larry Gold (University of Colorado) and modified as described below. The. . .

DETD . . . carried out largely as described in Chen & Gold, Biochemistry 33, 8746-8756 (1994). The template for synthesis of the starting ***RNA*** pool was based on a DNA oligonucleotide, 5'-CCCGGATCCTAGTTCACGATGCTGCAA-(N).sub.40-TTACGGTCTGAGAAAATATCCTCCC-3'(SEQ ID NO:27), where N indicates an equimolar mixture of A, G, C. . .

DETD This template preparation was incubated for 2-3 h at 37.degree. C. in a reaction mixture containing 475 U/ml T7 ***RNA*** polymerase, 40 mM Tris-HCl, pH 8.0, 12 mM MgCl.sub.2, 5 mM DTT, 1 mM spermidine, 0.002% Triton X-100, 4% PEG. . . was continued for 30 minute. The resulting RNAs were fractionated by 10% urea-PAGE and gel slices containing full-length 92 nucleotide ***RNA*** were excised, crushed, mixed with 0.5 ml TE, 0.5 ml phenol, and 10 .mu.l 10% sodium dodecyl sulfate, and tumbled overnight at 4.degree. C. to extract ***RNA*** . The supernatant was collected and ***RNA*** was precipitated with NaOAc and EtOH as described above. ***RNA*** was dissolved in TE, denatured at 100.degree. C. for 2 minute, and renatured by adjusting to 5 mM MgCl.sub.2, cooling rapidly to 0.degree. C., and incubating for 30 minute. The amount of ***RNA*** recovered was estimated by liquid scintillation counting.

DETD To perform in vitro selections, the ***RNA*** pool was mixed with purified Ku protein under conditions for complex binding. ***RNA*** and Ku protein were incubated for 30 minute at room temperature in a buffer containing 25 mM Tris-HCl, pH 7.9,. . . glycerol, 5 mM MgCl.sub.2, 0.5 mM DTT, and 0.01% Tween 20 in a final volume of 50-100 .mu.l. Concentrations of ***RNA*** , Ku protein, and KCl were

were isolated by electrophoresis on a 5% non-denaturing polyacrylamide gel containing 25 mM Tris-HCl, pH 8.3, 190 mM glycine,. . . over a nitrocellulose filter (Millipore 25-.mu.m HAWP), which was washed twice with 5 ml of 25 mM Tris-HCl, pH 7.9. ***RNA*** was eluted from the polyacrylamide gel or the filter by tumbling in a mixture of TE, phenol, and SDS as. . .

DETD Once the enriched ***RNA*** molecules were separated from the isolated complexes, they were amplified by reverse transcription followed by polymerase chain reaction. The complexity. . .

DETD When additional rounds of selection were performed, purified Ku protein (from HeLa cell nuclear extracts) was mixed with the ***RNA*** pool and allowed to bind. Ku- ***RNA*** complexes were isolated using either a nitrocellulose filter binding or an electrophoretic mobility shift assay (EMSA). ***RNA*** that was bound to Ku protein was isolated, amplified by RT-PCR, and again enriched based on the ability to bind to Ku protein. The selected ***RNA*** was reverse-transcribed by incubating in a reaction mixture containing 50 mM Tris-HCl, pH 8.3, 60 mM NaCl, 6 mM Mg(OAc)2,. . . Selection conditions used for each round of selection are given in Table 2. To maintain the stringency of selection, the ***RNA*** :protein ratio, the KCl concentration, or both were progressively increased as shown in Table 2. Two independent selections were conducted using different batches of starting ***RNA*** .

DETD After six or seven rounds of selection, cDNA was synthesized from the final ***RNA*** pool and amplified by 5 cycles of PCR using primer 1 and 2. The product was digested with Bam HI. . .

DETD

TABLE 2

Summary of the Selection Parameters

Selection I

SELEX Round	Input ***RNA*** (nM)	Ku protein (nM)	KCl.sup.b (mM)	Selection.sup.c Method
1	1305	64.1	50	NCFA
2	612.5	24.3	50	EMSA
. . .				EMSA
4	1130	42	120	EMSA
5	2320	64	150	EMSA
6	142	3.2	160	EMSA

Selection II

SELEX Round	Input ***RNA*** (nM)	Ku protein (nM)	KCl (mM)	Selection Method
1	1330	53	120	EMSA
2	1660	42	120	EMSA
. . .			160	EMSA
6	2710	18.6	160	EMSA
7	436	2.4	160	EMSA

.sup.aThe ratio of Ku protein to input ***RNA*** was gradually decreased in order to increase the stringency.

.sup.bSalt concentration was increased to maintain high stringency.

.sup.cNitrocellulose. . .

DETD . . . PK enzymatic activity. The ability of Ku protein to bind tightly to specific RNAs is consistent with a role for ***RNA*** in the regulation of Ku protein activity or intranuclear localization. Additionally, the identification of diverse RNAs that bind avidly to. . .

DETD Binding of ***RNA*** and DNA to Ku protein was measured using EMSA as shown by the representative Ku protein- ***RNA*** binding curves after successive rounds of SELEX in FIG. 1. Binding curves were obtained with EMSA followed by a nonlinear least squares fit of the data. The ***RNA*** pools in FIG. 1 were transcribed from templates prior to the first round (nonselected), and after the fourth (4th), and the sixth (6th) rounds of SELEX using T7 ***RNA*** polymerase as described in example 2. A twenty-one base pair double-stranded DNA was end-labeled with T4 polynucleotide kinase. The DNA. . .

DETD Ku protein bound to nonselected ***RNA*** with an apparent average K.sub.d of 24 nM. This binding was somewhat stronger than expected, given that previous work had found that Ku protein had little ability to interact with tRNA or synthetic ***RNA*** polymers (Mimori & Hardin, J. Biol. Chem. 261, 10375-10379 (1986)). Pooled ***RNA*** tested after the 4th and 6th round of SELEX showed an increased ability to bind

bound with an apparent K.sub.d of about 0.3 nM, which was comparable to the binding seen with a double-stranded DNA oligonucleotide under similar conditions. Although the average affinity of the selected ***RNA*** was only 75-fold greater than for the nonselected pool, subsequent experiments revealed clear functional differences, as only the selected RNAs. . .

DETD Individual aptamer RNAs were synthesized by T7 ***RNA*** polymerase using linearized plasmid template. Both radiolabeled ***RNA*** and nonradiolabeled ***RNA*** were prepared using a MEGA shortscript T7 kit (Ambion) with 8 .mu.g linearized plasmid template. The ***RNA*** was gel-purified, heated, and refolded as described in example 1, and the final concentration was determined spectrophotometrically. Secondary structures were. . . Acad. Sci. USA 86, 7706-7710 (1989); Jaeger et al., Meth. Enzymol. 183, 281-306 (1990)) as implemented on the mfold server www.ibc.wustl.edu/%7Ezuker/ ***rna*** /form 1.cgi).

DETD Synthesis of ***RNA*** Containing the ***TAR*** Sequence

DETD Pools of ***RNA*** at various stages of selection were also characterized directly for their ability to bind to Ku protein in an electrophoretic. . . mobility shift assay. For comparison of the aptamer RNAs binding affinity with other known RNAs, these assays also included a ***HIV*** ***TAR*** ***RNA*** , which was synthesized by T7 ***RNA*** polymerase as described in the preceding section. This ***RNA*** consisted of the following ***HIV*** -derived sequence: GGGUCUCUCUGGUUAGACCAGAUCUGAGCCUGGGAGCUCUCUGGC UAACUAGGGAACCC(SEQ ID NO:31).

DETD ***TAR*** -containing RNAs have previously been reported to bind selectively to Ku protein (Kaczmarski & Khan, Biochem. Biophys. Res. Commun. 196, 935-942. . .

DETD Binding of the ***TAR*** ***RNA*** was measurable, but weak, under the conditions of the experiments. These results confirm that ***TAR*** -containing ***RNA*** has some ability to bind to Ku protein, but indicate that the binding is not particularly strong, relative to other. . .

DETD After six and seven rounds of SELEX (Selection I and II in Table 2, respectively), the pooled ***RNA*** was reverse-transcribed, PCR-amplified, and cloned into plasmid vectors. 82 clones were isolated, including 63 from Selection I and 19 from. . .

DETD . . . designated SEQ ID NO:20) (wherein N may be A, C, G or T), which was perfectly conserved in three independent ***RNA*** sequences and partially conserved in four others. A second motif, AMAUGA (herein designated SEQ ID NO:21) (wherein M may be. . . partially overlaps the second, had the sequence AACUUCGA (bases 31-38 of SEQ ID NO:15). This motif was present in one ***RNA*** and partially conserved in four others. Several RNAs fell into hybrid classes containing two of the three motifs. Subsequent analysis. . .

DETD Inhibition of DNA-PK Activity by Different ***RNA*** Aptamers

DETD Binding of the ***RNA*** aptamers to Ku protein to determine if they are capable of regulating Ku protein activity was then measured. One of. . .

DETD . . . block the ability of Ku protein to activate DNA-PK. Each of the nineteen selected aptamers, as well as the nonselected ***RNA*** pool, was tested in a DNA-PK assay. Each ***RNA*** was prepared by in vitro transcription and added to a DNA-PK phosphorylation assay.

DETD . . . (EPPLSQEAFADLLWKK(SEQ ID NO:32)) containing a DNA-PK phosphorylation site from p53 (underlined), and the indicated amounts (8, 16, 32 nM) of ***RNA*** aptamer. Native DNA-PKcs were purified from HeLa cell nuclear extracts as described in example 2. In FIG. 2A, DNA-PK activity is expressed as a percentage of activity in the absence of ***RNA*** . Values shown are averages of duplicate reactions with standard deviations indicated. Background phosphorylation in the absence of DNA has been. . .

DETD The effect on DNA-PK activity was measured, relative to control reactions with no added ***RNA*** . Nonselected ***RNA*** had no effect on DNA-PK activity (FIG. 2A). Of the selected RNAs, seven inhibited DNA-PK activity by 85% or more. . .

DETD . . . in the presence of DNA. However, under these conditions, the DNA-PK was already highly active and a weak ability of ***RNA*** to activate DNA-PK might have gone undetected. Indeed, displacement of DNA by a weakly activating ***RNA*** may have resulted in partial inhibition of DNA-PK activity.

DETD . . . described above in the presence or absence of 0.25 nM double-stranded DNA, as indicated. Reactions contained various amounts of aptamer ***RNA*** (8, 16, 32 nM).

DETD When neither ***RNA*** nor DNA was present, DNA-PK had a low level of basal activity, as expected. The addition of double-stranded DNA increased. . .

DETD In principle, an ***RNA*** that binds to Ku protein could inhibit DNA-PK either by blocking the binding of Ku protein to DNA or by. . .

DETD . . . double stranded DNA (1 nM) oligonucleotide in the presence or absence of various amounts (8, 16, 32 nM) of nonradiolabeled ***RNA*** . The incubation conditions were 25 mM Tris-HCl pH 7.9, 0.5 mM EDTA, 10% glycerol, 5 mM MgCl.sub.2, 0.5 mM DTT,. . . inhibitors of DNA-PK enzyme activity also proved to be effective inhibitors of Ku protein-DNA binding activity (FIG. 2B). By contrast, ***RNA*** from the nonselected pool had no effect on Ku protein-DNA interaction (FIG. 2B). There was a rough correlation between the. . .

DETD . . . mapped, it is likely that it contains basic residues that are capable of electrostatic interactions with the phosphate backbone of ***RNA*** and DNA.

DETD To further explore the relationship between ***RNA*** and DNA binding sites in Ku protein, Electrophoretic Mobility Shift Assays (EMSAs) were performed comparing radiolabeled aptamer ***RNA*** (1 nM) and radiolabeled twenty-one base pair double stranded DNA (1 nM) probes. Cross-competition between ***RNA*** aptamers and DNA for Ku protein binding were found. It was discovered that Ku protein forms stable complexes with both types of probes, and that the complexes with ***RNA*** and DNA have similar electrophoretic mobilities. Both of the RNAs that were tested, #7-3 and #SC9, competed with DNA for binding to Ku protein. ***RNA*** #7-3 is a more effective competitor than ***RNA*** #SC9, consistent with the results of nitrocellulose filter binding assays. In the reciprocal experiment, nonradiolabeled DNA fragments competed with labeled ***RNA*** for binding to Ku protein. As controls, DNA and each ***RNA*** were shown to effectively self-compete for binding to Ku protein, confirming the fidelity of the analysis.

DETD Notably, neither the DNA nor the ***RNA*** competitors induced the formation of supershifted complexes. The absence of supershifted complexes provides additional evidence that ***RNA*** and DNA cannot bind simultaneously to Ku protein, but rather, bind competitively to the same site.

DETD . . . .mu.g protein) and other components as in FIG. 2. Reactions were performed in the presence of various amounts of aptamer ***RNA*** (250, 500, 1000 nM) as indicated. The assays were as described 20 above except a higher amount of DNA was. . . 100 to 142.1 .mu.M p53 was used. DNA-PK activity is expressed as a percentage of activity in the absence of ***RNA*** . Values shown are averages of duplicate reactions with standard deviations as indicated. Four different aptamer RNAs, all of which had. . . been shown to inhibit purified DNA-PK, were tested in the crude extract. All four reduced enzyme activity to near-basal levels. ***RNA*** from the nonselected pool had no effect at concentrations up to 1000 nM (FIG. 3, lanes 5-7). These results demonstrate. . .

DETD . . . (0.38 nM) under conditions for forming complexes for 30 minutes at room temperature. The complexes were challenged with either aptamer ***RNA*** or ***RNA*** from the nonselected pool. Incubation was then continued and the amount of Ku-DNA complexes remaining were measured by electrophoretic mobility. . .

DETD . . . the presence of aptamer #7-3. In contrast, the amount of Ku protein-DNA complexes remained almost unchanged when challenged with nonselected ***RNA*** . This result demonstrates that the aptamer ***RNA*** can compete with double-stranded DNA for the binding of Ku protein even if the aptamer ***RNA*** is added after Ku-DNA complexes have formed.

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Set     Items   Description
S1         23   NUCLEOLAR (S) RNA (S) TAR
S2         13   RD (unique items)
S3         34   NUCLEOLAR AND TAR AND RNA
S4         22   RD (unique items)
>>>KWIC option is not available in file(s): 399
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4/3,K/1 (Item 1 from file: 5)

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DIALOG(R)File   5:Biosis Previews(R)
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14021055   BIOSIS NO.: 200300015084
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A *nucleolar* *TAR* decoy inhibitor of HIV-1 replication.

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A *nucleolar* *TAR* decoy inhibitor of HIV-1 replication.

...ABSTRACT: critical regulatory factor in HIV-1 gene expression. It mediates the transactivation of transcription from the HIV-1 LTR by binding to the transactivation response (*TAR*) element in a complex with cyclin T1. Because of its critical and early role in HIV gene expression, Tat and its interaction with the *TAR* element constitute important therapeutic targets for the treatment of HIV-1 infection. Based on the known *nucleolar* localization properties of Tat, we constructed a chimeric small *nucleolar* *RNA*-*TAR* decoy that localizes to the nucleoli of human cells and colocalizes in the nucleolus with a Tat-enhanced GFP fusion protein. When the chimeric *RNA* was stably expressed in human T lymphoblastoid CEM cells it potently inhibited HIV-1 replication. These results demonstrate that the *nucleolar* trafficking of Tat is critical for HIV-1 replication and suggests a role for the nucleolus in HIV-1 viral replication.

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DESCRIPTORS:
  ...BIOSYSTEMATIC NAMES: DNA and *RNA* Reverse Transcribing Viruses,
    Viruses, Microorganisms
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4/3,K/2 (Item 2 from file: 5)

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DIALOG(R)File   5:Biosis Previews(R)
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07779643   BIOSIS NO.: 000092083014
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HETEROLOGOUS BASIC DOMAIN SUBSTITUTIONS IN THE HIV-1 TAT PROTEIN REVEAL AN ARGININE-RICH MOTIF REQUIRED FOR TRANSACTIVATION

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FULL JOURNAL NAME: EMBO (European Molecular Biology Organization) Journal
CODEN: EMJOD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH
```

...ABSTRACT: Tat protein coded by HIV-1 is a unique eukaryotic transactivator. It activates gene expression from the viral LTR by its interaction with a nascent *RNA* element (*TAR*) located at the 5 end of all HIV-1 transcripts. Tat appears to bind to its target *RNA* structure

in a highly sequence-specific manner. The *TAR*-binding activity of Tat has been localized in an Arg-rich basic domain located between residues 49 and 57 of the Tat protein. We have carried out domain substitution studies with heterologous basic domains which are also implicated in *RNA* binding. Here, we report that a 19 or a 12 amino acid region from the N-terminus of HTLV-I Rex can functionally substitute for...

...to the nucleus and nucleolus. The chimeric Tat proteins containing the basic domains of Rex and the N proteins are also localized in the nuclear/*nucleolar* region. Since the functionally inative Tat - N chimeric proteins are still efficiently targeted to the nuclear/ *nucleolar* compartments, our results suggest that nuclear/*nucleolar* localization alone is not sufficient for transactivation and demonstrate a direct role for the Arg motif in Tat function other than that required for nuclear/*nucleolar* localization.

4/3,K/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07320762 BIOSIS NO.: 000090100662
A BULGE STRUCTURE IN HIV-1 *TAR* *RNA* IS REQUIRED FOR TAT BINDING AND TAT-MEDIATED TRANS-ACTIVATION
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JOURNAL: GENES DEV 4 (8). 1990. 1365-1373. 1990
FULL JOURNAL NAME: Genes & Development
CODEN: GEDEE
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

A BULGE STRUCTURE IN HIV-1 *TAR* *RNA* IS REQUIRED FOR TAT BINDING AND TAT-MEDIATED TRANS-ACTIVATION

...ABSTRACT: immunodeficiency virus type 1 (HIV-1) trans-activates viral gene expression and is obligatory for virus replication. Tat function is mediated through a sequence termed *TAR* that comprises part of the 5'-noncoding region of all HIV-1 mRNAs. This region forms a stable stem-loop structure in vitro. Recent evidence indicates that Tat binds directly to the *TAR* *RNA* sequence, and this binding is independent of the nucleotide sequence in the loop but dependent on the integrity of the upper stem. We used the...

...structure specificity of this interaction and its correlation with Tat trans-activation. We show that a 3-nucleotide bulge structure (positions +23 to +25) in *TAR* *RNA* is important for both Tat interaction with *TAR* *RNA* and Tat-mediated trans-activation of gene expression. Single base substitutions at position +23 that impair Tat-mediated trans-activation in vivo also reduce binding of Tat to *TAR* in vitro, suggesting that the first uridine residue in the bulge is the critical base for both functions. In contrast, mutations in the loop (positions...

...reduce Tat-mediated trans-activation, had no effect on Tat binding. We also show that a Tat peptide that includes the basic region required for *nucleolar* localization binds to *TAR* *RNA* with the same specificity as the full-length protein. We conclude that Tat binding to *TAR* is necessary but not sufficient by itself to account for trans-activation.

4/3,K/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07294844 BIOSIS NO.: 000090074731
A TRANSDOMINANT TAT MUTANT THAT INHIBITS TAT-INDUCED GENE EXPRESSION FROM

THE HUMAN IMMUNODEFICIENCY VIRUS LONG TERMINAL REPEAT
AUTHOR: PEARSON L; GARCIA J; WU F; MODESTI N; NELSON J; GAYNOR R
AUTHOR ADDRESS: DIV. HEMATOL.-ONCOL., DEP. MED., UNIV. OF CALIFORNIA-LOS ANGELES, SCH. OF MED., LOS ANGELES, CALIF. 90024.
JOURNAL: PROC NATL ACAD SCI U S A 87 (13). 1990. 5079-5083. 1990
FULL JOURNAL NAME: Proceedings of the National Academy of Sciences of the United States of America
CODEN: PNASA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

...ABSTRACT: virus (HIV) gene expression is dependent on specific regulatory regions in the long terminal repeat. These regions include the enhancer, SP1, "TATA," and trans-activating (*TAR*) regions. In addition, viral regulatory proteins such as tat and rev are important in regulating HIV gene expression. The mechanism of tat activation remains the...

...was localized predominantly to the nucleolus, .DELTA.tat was present in both the nucleus and cytoplasm, suggesting that it may inhibit tat function by preventing *nucleolar* localization. Transdominant mutants of tat may have a role in potentially inhibiting HIV gene expression.
DESCRIPTORS: DNA MESSENGER *RNA* TAT PROTEIN

4/3,K/5 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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07360640 Genuine Article#: 155QX No. References: 81
Title: The yeast Saccharomyces cerevisiae YDL112w ORF encodes the putative 2'-O-ribose methyltransferase catalyzing the formation of Gm18 in tRNAs
Author(s): Cavaille J; Chetouani F; Bachellerie JP (REPRINT)
Corporate Source: UNIV TOULOUSE 3,CNRS, LBME, 118 ROUTE NARBONNE/F-31062 TOULOUSE//FRANCE/ (REPRINT); UNIV TOULOUSE 3,CNRS, LBME/F-31062 TOULOUSE//FRANCE/
Journal: RNA-A PUBLICATION OF THE RNA SOCIETY, 1999, V5, N1 (JAN), P66-81
ISSN: 1355-8382 Publication date: 19990100
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

Abstract: The protein sequences of three known *RNA* 2'-O-ribose methylases were used as probes for detecting putative homologs through iterative searches of genomic databases. We have identified 45 new positive Open ...

...organism, is considerably larger than its Escherichia coli functional homolog spoU (1,436 amino acids vs. 229 amino acids), or any known or putative prokaryotic *RNA* ribose methyltransferase. Homologs found in human (TRP-185 protein), Caenorhabditis elegans and Arabidopsis thaliana also exhibit a very long N-terminal extension not related to ...
...Identifiers--SMALL *NUCLEOLAR* RNAS; ADENOSYLMETHIONINE-DEPENDENT METHYLTRANSFERASES; TRANSACTIVATING REGION *RNA*; HIV-1 *TAR* *RNA*; RIBOSOMAL-*RNA*; ESCHERICHIA-COLI; SEQUENCE MOTIFS; MESSENGER-*RNA*; SUBNUCLEAR LOCALIZATION; PSEUDOURIDINE SYNTHASE

4/3,K/6 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.

06685289 Genuine Article#: ZK562 No. References: 35
Title: *Nucleolar* protein p120 contains an arginine-rich domain that binds to ribosomal *RNA*
Author(s): Gustafson WC; Taylor CW; Valdez BC; Henning D; Phippard A; Ren Y ; Busch H; Durban E (REPRINT)

Corporate Source: BAYLOR COLL MED,DEPT PHARMACOL, 1 BAYLOR PLAZA/HOUSTON//TX/77030 (REPRINT); BAYLOR COLL MED,DEPT PHARMACOL/HOUSTON//TX/77030
Journal: BIOCHEMICAL JOURNAL, 1998, V331, 2 (APR 15), P387-393
ISSN: 0264-6021 Publication date: 19980415
Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON, ENGLAND W1N 3AJ
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

Title: *Nucleolar* protein p120 contains an arginine-rich domain that binds to ribosomal *RNA*

...Abstract: Northwestern blotting procedure this study shows that recombinant p120 binds to an rRNA fragment in vitro with a dissociation constant of 4 nM. The specific *RNA*-binding region of p120 (residues 1-57) was identified with glutathione S-transferase-fused p120 deletion constructs and Northwestern blotting procedures. This *RNA*-binding region of p120, which includes the *nucleolar* localization signal of p120, is similar to the arginine-rich *RNA*-binding regions found in other *RNA*-binding proteins such as HIV Rev and Tat. Experiments in viuo with HeLa cell *nucleolar* extracts showed that p120 was associated with the 60-80 S pre-ribosomal particles. This association is disrupted by treatment with either RNase A or...

...Identifiers--POLYACRYLAMIDE-GEL ELECTROPHORESIS; HEPATOMA ASCITES-CELLS; RAT-LIVER; *TAR* *RNA*; PROLIFERATION; LOCALIZATION; RECOGNITION; NUCLEOPHOSMIN/B23; IDENTIFICATION; HOMOLOGY

4/3,K/7 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05805295 Genuine Article#: WY721 No. References: 36

Title: The post-transcriptional regulator Rev of HIV: Implications for its interaction with the *nucleolar* protein B23

Author(s): Miyazaki Y (REPRINT) ; Nosaka T; Hatanaka M
Corporate Source: KYOTO UNIV,INST VIRUS RES, SAKYO KU/KYOTO 606//JAPAN/ (REPRINT); SHIONOGI INST MED SCI,/OSAKA 566//JAPAN/
Journal: BIOCHIMIE, 1996, V78, N11-12, P1081-1086
ISSN: 0300-9084 Publication date: 19960000
Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER, 141 RUE JAVEL, 75747 PARIS CEDEX 15, FRANCE
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

Title: The post-transcriptional regulator Rev of HIV: Implications for its interaction with the *nucleolar* protein B23

...Abstract: is controlled by trans-acting proteins. HIV-1 encodes several regulatory proteins, including two essential transactivations for viral replication, Rev and Tat. Both Rev and *Tar* have a *nucleolar* targeting signal and are actually located predominantly in the nucleoli. Within the nucleoli, Rev is localized to the combined regions of the dense fibrillar (DFC) and the granular (GC) components Tat does not colocalize precisely with any *nucleolar* component tested, but partly overlaps regions of the DFC and the GC. Regions of both Rev and Tat are overlapped by the distribution of the major *nucleolar* protein B23. Overexpression of Rev causes *nucleolar* ballooning and general structural deformity with aberrant accumulation of rRNAs, whereas Tat does not have that effect. B23 is markedly accumulated in those nucleoli deformed by Rev. Components of the *nucleolar* DFC, GC, and fibrillar center domains are not accumulated but dispersed in a few small spots or larger patches within the enlarged nucleoli. Cytophotometric DNA...

4/3,K/8 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2003 Inst for Sci Info. All rts. reserv.

02573723 Genuine Article#: LM272 No. References: 38

Title: *TAR* LOOP-DEPENDENT HUMAN-IMMUNODEFICIENCY-VIRUS TRANSACTIVATION REQUIRES FACTORS ENCODED ON HUMAN CHROMOSOME-12
Author(s): HART CE; GALPHIN JC; WESTHAFER MA; SCHOCHETMAN G
Corporate Source: CTR DIS CONTROL & PREVENT,DIV HIV AIDS,LAB INVEST BRANCH D12,1600 CLIFTON RD/ATLANTA//GA/30333
Journal: JOURNAL OF VIROLOGY, 1993, V67, N8 (AUG), P5020-5024
ISSN: 0022-538X
Language: ENGLISH Document Type: NOTE (Abstract Available)

Title: *TAR* LOOP-DEPENDENT HUMAN-IMMUNODEFICIENCY-VIRUS TRANSACTIVATION REQUIRES FACTORS ENCODED ON HUMAN CHROMOSOME-12
Abstract: The trans-activator response region (*TAR*) *RNA* in the human immunodeficiency virus type 1 (HIV-1) and HIV-2 long terminal repeat forms stem-loop secondary structures in which the loop sequence is essential for trans activation. We investigated how the HIV trans-activation mechanism encoded on human chromosome 12 relates to the *TAR* *RNA* loop-dependent pathway. DNA transfection experiments showed that trans activation in human-hamster hybrid cells with the single human chromosome 12 and human T-cell lines was highly dependent on the native sequences of the HIV-1 *TAR* loop and the HIV-2 5' *TAR* loop. In nonhuman cell lines or hybrid cells without chromosome 12 that supported trans activation, the cellular mechanism was independent of the HIV-1 *TAR* loop and the response to mutations in the HIV-2 *TAR* loops differed from that found in human T-cell lines and human-hamster hybrid cells with chromosome 12. Our results suggest that the human chromosome 12 mechanism interacts directly with the *TAR* *RNA* loop or indirectly by regulating *TAR* *RNA*-binding proteins.
...Identifiers--MEDIATED TRANSACTIVATION; GENE-EXPRESSION; BINDING PROTEIN; NUCLEAR-PROTEIN; *RNA* SEQUENCES; RODENT CELLS; HIV-1 LTR; TYPE-1; REGION; ELEMENT
Research Fronts: 91-0190 001 (HUMAN-IMMUNODEFICIENCY-VIRUS TYPE-1; CHRONICALLY INFECTED CD8+ CELLS; COMPATIBILITY OF REV GENE ACTIVITY)
91-2472 001 (*NUCLEOLAR* ORGANIZER REGIONS; CELL CYCLE-DEPENDENT PHOSPHORYLATION OF HUMAN DNA POLYMERASE-ALPHA; ABERRANT KI-67 EXPRESSION)
91-3227 001 (*RNA* SECONDARY STRUCTURE; PRIMARY SEQUENCE; RHO-INDEPENDENT ESCHERICHIA-COLI TRANSCRIPTION TERMINATORS)
91-5983 001 (TRANSCRIBED REGION OF THE HUMAN C-FOS GENE; TISSUE-SPECIFIC ENHANCER ELEMENTS...

4/3,K/9 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01613878 Genuine Article#: HL816 No. References: 20
Title: SPECIFIC BINDING OF ARGININE TO *TAR* *RNA*
Author(s): TAO JS; FRANKEL AD
Corporate Source: WHITEHEAD INST BIOMED RES,9 CAMBRIDGE CTR/CAMBRIDGE//MA/02142
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1992, V89, N7 (APR 1), P2723-2726
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

Title: SPECIFIC BINDING OF ARGININE TO *TAR* *RNA*
...Abstract: arginine residue within the basic region of the human immunodeficiency virus Tat protein mediates specific binding of Tat peptides to a three-nucleotide bulge in *TAR* *RNA*. It has been proposed that arginine recognizes *TAR* by forming a network of hydrogen bonds with two structurally distinct phosphates, an interaction termed the "arginine fork." Here it is shown that L-arginine blocks the Tat peptide/*TAR* interaction, whereas L-lysine and analogs of arginine that remove specific hydrogen bond donors do not. Experiments using an L-arginine affinity column demonstrate that arginine and the Tat peptides bind to the same site in *TAR*. Modification of two phosphates located at the junction of the double-stranded stem and bulge and modification of two adenine N7

groups in base-paired regions of *TAR* interfere with specific arginine binding. The results emphasize the importance of *RNA* structure in *RNA*-protein recognition and provide methods to identify arginine-binding sites in RNAs.
Research Fronts: 90-1803 001 (5S RIBOSOMAL-*RNA* SEQUENCES; U3 SMALL *NUCLEOLAR* RIBONUCLEOPROTEIN FUNCTIONS; BACTERIAL EVOLUTION)
90-8119 001 (ESCHERICHIA-COLI METHIONYL-TRANSFER *RNA*-SYNTHETASE; MUTUALLY EXCLUSIVE SETS OF SEQUENCE MOTIFS; FRAMEWORK FOR UNDERSTANDING HOMEODOMAIN-DNA INTERACTIONS)

4/3,K/10 (Item 1 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04517797 H.W. WILSON RECORD NUMBER: BGSA01017797 (USE FORMAT 7 FOR FULLTEXT)
Viruses and interferons.
Sen, Ganes C
Annual Review of Microbiology v. 55 (2001) p. 255-81
SPECIAL FEATURES: bibl il ISSN: 0066-4227
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 12173

(USE FORMAT 7 FOR FULLTEXT)

...ABSTRACT: cell signaling used by many cytokines and growth factors. Surprisingly, some of the same genes can also be induced directly by viruses and double-stranded *RNA*, a common viral by-product. Some of the interferon-induced proteins have novel biochemical properties and some are inactive as such but can be activated by double-stranded *RNA* produced during virus infection. Finally, almost all viruses have evolved mechanisms to evade the interferon system by partially blocking interferon synthesis or interferon action. Thus...

TEXT:
Key Words antiviral effects, double-stranded *RNA*, interferon-induced proteins, transcriptional regulation, Jak-STAT pathway

INTRODUCTION TO THE INTERFERON SYSTEM
Interferons (IFNs) are the first known members of the important biological regulatory...

...IFN-induced proteins remain unknown, some of the proteins that have been analyzed carry out interesting functions. Moreover, two such proteins use double-stranded (ds) *RNA* as a cofactor for their enzymatic activity, providing the first biochemical function of dsRNA.

In the context of host-virus infection, regulation of components of... activation of IFN-a/b genes is mediated by complex pathways involving many transcription factors. A well-characterized patway is triggered by viral double-stranded *RNA* expressed in infected cells. Other lesser-known pathways that are activated by viral interactions with cellular receptors or by viral proteins must exist as well...

...the cytoplasm and are activated by phosphorylation. NFkB activation involves activation of the IkB kinase complex and the dsRNA-dependent protein kinase, PKR (protein kinase, *RNA*-activated) (12). P38 and JNK (Jun N-terminal kinase) kinases are also activated by MEKK1 (microtubule-associated protein, extracellular response kinase kinase) (39). Complete characterization...length of double-stranded region is good enough for activating them maximally (72). Consequently, many viral RNAs with partial ds structures, such as adenoviral VAI *RNA*, EB viral EBER *RNA*, and HIV-1 *TAR* *RNA*, can activate them (74). The small enzymes are tetramers and the medium isozymes are dimers; their oligomerization is needed for their enzyme activity. The catalytic...c-Myc, and Rb (15). The

inhibition of c-Myc activity is mediated by blocking its association with Max (94). Another member, P204, is a *nucleolar* protein that inhibits ribosomal *RNA* transcription by binding to an essential transcription factor, UBF-1 (50). There are indications that these proteins may have cellular functions beyond the IFN system...EMCV) (26). This effect requires enzymatically active proteins and the presence of RNaseL, indicating the need for 2-5(A) production and activated RNaseL-mediated *RNA* degradation (33). Viral dsRNA is the likely activator of 2-5(A) synthetases because they coimmunoprecipitate to form an enzymatically active complex (28). Although the...signaling are HBV terminal protein, EBV EBNA1 and EBNA2 proteins, HPV E7 protein, polyoma virus large T, and HCMV and HHV8 proteins (7, 27). Numerous *RNA* viruses also interfere with IFN signaling. These include influenza virus, ebola virus, Sendai virus, SV5, bovine respiratory syncytial virus (BRSV), parainfluenza virus, and hepatitis C...encode RNAs that have partial double-stranded structures, and at high concentrations they block PKR activation by authentic dsRNA. Such viral RNAs include adenoviral VAI *RNA*, EBV EBER *RNA*, and HIV-1 *TAR* *RNA* (7, 27). Vaccinia virus E3L protein, influenza virus NS1 protein (5), and reovirus sigma 3 protein bind dsRNA and thus can block PKR activation. The...

...through unknown mechanisms. HSV1 encodes two proteins that can interfere with PKR activation or action (86). One of these proteins is Us11, a virally encoded *RNA*-binding protein (9). The other is the g1 34.5 protein. The carboxyl terminus of this protein binds to the cellular protein phosphatase 1a and...

...antiproliferative activity of IFN-a. Differential IFN-a sensitivity of different clonal derivatives of Daudi cells has been correlated with the abundance of a viral *RNA* that has considerable double-stranded structure (24). This *RNA* may activate PKR, leading to translation inhibition, c-Myc repression, and NFkB activation, causing cell growth inhibition. IFN-g may promote cell proliferation or apoptosis...grants from the National Institutes of Health.

Figure 1 Interactions between viruses and the interferon system. Viruses enter the cell, get uncoated, and synthesize viral *RNA* and proteins. Often viral dsRNA is also produced. dsRNA can signal to the promoter of interferon (IFN)-a/b genes by activating the transcription factors...

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4/3,K/11 (Item 2 from file: 98)

04255630 H.W. WILSON RECORD NUMBER: BGSA00005630 (USE FORMAT 7 FOR FULLTEXT)

Lentivirus replication and regulation.

AUGMENTED TITLE: review

Tang, Hengli
Kuhen, Kelli L; Wong-Staal, Flossie

Annual Review of Genetics v. 33 (1999) p. 133-70
SPECIAL FEATURES: bibl il ISSN: 0066-4197
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 17942

(USE FORMAT 7 FOR FULLTEXT)

TEXT:

... immunodeficiency virus, transcriptional regulation, posttranscriptional control, downregulation

INTRODUCTION

GENOMIC STRUCTURES OF LENTIVIRUSES

Lentiviruses comprise one of three subfamilies of retroviruses, which also include the oncoviruses (*RNA* tumor viruses) and spuma (or foamy) viruses. Members of all three subfamilies are able to infect humans, and are associated with a spectrum of pathogenic...

...readers to the excellent recent text on Retroviruses (42). Rather, we give a broad outline here for the purpose of comparison with the lentiviruses. The *RNA* genome of retroviruses is flanked by two short, redundant (R) sequences at both termini. These are adjacent to unique sequences, U5 and U3, found at the 5' and 3' ends, respectively. The defining feature of a retrovirus is its ability to convert its *RNA* genome to a DNA intermediate through the virally encoded reverse transcriptase. A subsequent reaction catalyzed by another viral enzyme, integrase, results in the integration of...

...coupled and only completely spliced mRNA species are exported to the cytoplasm. However, retroviruses need to bypass this regulation in order to utilize unspliced viral *RNA* both as messenger *RNA* for protein synthesis and as genomic *RNA* for progeny virions. Interestingly, lentiviruses and simpler retroviruses utilize different strategies to this same end, as detailed in a subsequent section.

The virion capsid contains a diploid genome of two (+) *RNA* strands. A small region, usually spanning the 3' end of the LTR and the 5' end of the gag gene, mediates specific binding to the...a natural host cofactor for HIV-1 integration (33).

TRANSCRIPTIONAL REGULATION

Regulation of HIV-1 transcription involves a complex interplay between cis-acting DNA and *RNA* elements present within the chromatin-associated proviral long terminal repeats (LTRs), cellular transcription factors (DNA level), and the viral trans-regulatory protein Tat (*RNA* level).

The HIV-1 LTR and Regulatory Elements The long terminal repeat (LTR) DNA, defined by the U3, R, and U5 regions, possesses multiple cis...

...3). The LTR can be divided into several discrete functional domains: the negative regulatory element (NRE), the enhancer, the basal promoter elements, the core promoter, *TAR* (Tat-activation response element), and the IST (inducer of short transcripts). Of note are two unique cis-acting elements: IST, a DNA element, functions as an enhancer that promotes the synthesis of abortive transcripts; and *TAR*, and *RNA* element present in the viral LTR and the 5' end of all viral mRNAs, is an enhancer that promotes the synthesis of processive transcripts.

Upstream...
...impacts transcription, as the loss of this region results in an increase in gene expression. This region has a negative effect on the rate of *RNA* initiation in vivo (145) and in vitro (181). The NRE has numerous candidate protein-binding sites and many of these are occupied, as observed by...boxes may regulate a switch from basal to activated gene expression (186). The TATA box functions to recruit a coordinated series of transcription factors and *RNA* polymerase II. Initiation of transcription is preceded by binding of TFIID (transcription factor IID), a pivotal factor in the basal transcription apparatus. This is followed by assembly of *RNA* polymerase II and the general initiation factors with the core promoter to form the preinitiation complex (27, 223). The formation of this complex is sufficient...

...the action of Tat in enhancing transcription (187). There is also a candidate binding site for CTF (CAAT-box transcription factor) in this region.

TAT/*TAR* Interaction Tat is a potent transactivator of gene expression for HIV-1. The most interesting and unique function of Tat is that it acts through an *RNA* target termed *TAR* located immediately 3' to the LTR transcription start site. The 59-nt *TAR* element forms a stable, partially base-paired stem-loop structure. This stable secondary structure has been confirmed by in vitro chemical and enzyme mapping and by NMR analysis (120). There are two critical sequence elements in *TAR* that are required for tat transactivation: a hexanucleotide loop at the tip and a three-nucleotide uracil-rich bulge within the stem (Figure 4). The...

...Tat span the bulge and adjacent sequences (37). Although not essential for binding of Tat in vitro, the loop is also important for the Tat-*TAR* interaction in vivo since any mutation in the loop sequence would abolish such interactions (70, 211).

Tat is a 14-kDa protein comprised of 86...

...protein possesses the activation domain. Residues 48 through 57 comprise an arg/lys-rich basic region that is responsible both for direct interaction with the *TAR* *RNA* target and for nuclear/*nucleolar* localization. The arginine at aa position 52 is critical for this specific association with *TAR*. The carboxy-terminal region, encoded by exon 2, is dispensable for function.

At the transcriptional level, the Tat/*TAR* interaction can potentially enhance or stabilize binding of transcription complexes required for both initiation and elongation of viral RNAs (134, 154). However, now the general consensus is that the primary function of Tat is to increase the processivity of *RNA* polymerase IIrather than stimulating transcription (205, 272). The increased initiation of transcription that is observed is probably a result of the higher rate of promoter...

...is a direct consequence of enhanced processivity. Much effort has been placed on identifying cellular cofactors of Tat. A number of cellular factors bind to *TAR*, but no clear role for any of these factors has been established in Tat regulation. Recently, a multisubunit cellular protein kinase called TAK (Tat-associated...

...was identified. TAK directly binds to the activation domain of Tat and, in turn, hyperphosphorylates the carboxyl-terminal domain (CTD) of the large subunit of *RNA* polymerase II (Pol II) (110, 267). The CTD possesses a heptad repeat consisting of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. Hypophosphorylated...

...of the TAK complex, directly binds to the activation domain of Tat (255). Furthermore, a functional complex requires the simultaneous binding of CycT1 to the *TAR* loop and Tat to the *TAR* bulge. CycT1 increases both the affinity and specificity of Tat for *TAR* (94). CycT1 was also identified as a regulatory subunit of Cdk9. As shown in Figure 4, the complex formation of Tat-CycT1 with *TAR* recruits CDk9 to the pol II transcription complex, ...factor encoded on human chromosome 12 (5, 105, 106). The observation that human cyclin T1 is encoded on chromosome 12,

enhances binding of Tat to *TAR* in vitro, and augments Tat transactivation in murine cells (255) suggests that the murine cyclin T protein is unable to support efficient Tat activation of...

...cysteine for tyrosine at position 261 in murine CycT restores zinc-dependent binding to Tat as well as high-affinity binding of the complex to *TAR* *RNA*. This single change was also found to be both necessary and sufficient for murine CycT to support high levels of Tat trans-activation in murine...

...stable expression of human CycT1 in murine cells was insufficient to overcome additional species-specific barriers to HIV-1 replication in murine cells.

Effects of *TAR* *RNA* and TAT on Translation The level of *TAR* *RNA* synthesized may activate or inhibit cellular repressors in different phases of virus replication. For example, high levels of *TAR*-containing *RNA* inhibit the interferon (IFN)-inducible, *RNA*-dependent protein kinase PKR (103). PKR is a pivotal mediator of the antiviral effects of IFN (216), and acts through phosphorylation of the a subunit of the translation factor eIF-2 (217). Many viruses have evolved strategies to inhibit PKR function (126). Thus, inhibition of PKR by *TAR* *RNA* would allow viral protein synthesis and a productive infection to proceed. In contrast, low levels of *TAR* *RNA* activate PKR (210), which may help to maintain the latent phase of virus infection in the host cell. In addition to *TAR*-containing RNAs, the Tat protein can also inhibit PKR kinase activity by competing with eIF-2a for binding to PKR (20, 162). Thus, it is conceivable that Tat and its viral *RNA* target sequence, *TAR*, can stimulate gene expression at the level of translation by inhibition of PKR function.

Downstream DNA Regulatory Elements In vitro and in vivo footprinting analyses...

...primary transcript upon transcription from the integrated proviral DNA, which serves as template for synthesis of gag and pol gene products as well as genomic *RNA* for encapsidation into progeny virions. It also serves as the precursor mRNA for further processing to generate multiple spliced mRNAs (244). Thus, retroviruses defy the...

...production. In addition to mediating nuclear export of unspliced mRNA, Rev has also been proposed to function through inhibition of splicing of the full-length *RNA* (32, 131) or increasing the efficiency of translation of the late gene products (6).

REV-RRE Interaction Rev of HIV-1 is a 116-amino activation domain. The N-terminal half of Rev contains sequences that are important for nuclear localization, multimerization, and *RNA*-binding. The Rev Response Element (RRE) binding site of Rev overlaps with the nuclear localization signal (NLS). Unlike the classical lysine-rich NLS, the Rev...

...b for nuclear import (109, 242). The activation domain at the carboxyl terminus of Rev contains a nuclear export signal (NES) that accesses a cellular *RNA* export pathway different from that of cellular mRNA (72). Mutations in this domain abolish the ability of Rev to shuttle between the cytoplasm and the...

...phenotype and are capable of repressing wild-type Rev function as well as HIV replication (149).

The RRE of HIV-1 is a highly structured *RNA* element that is present in the singly spliced env mRNA and the full-length genomic *RNA*. It serves as a high-affinity binding site of Rev on these *RNA* (150). Rev interacts with RRE in a multimerized form, directing RRE-containing *RNA* to a nuclear export pathway specified by its leucine-rich NES (activation domain). The Rev-RRE interaction has been studied extensively, both in vitro and in vivo. Rev binds to in vitro transcribed RRE *RNA* with high affinity and specificity. RRE contains extensive secondary structure, including several stem loops. The second stem from the 5' end, SLII, is the initial and primary binding site for Rev (11, 13). When an antibody directed against Rev was used to immunoprecipitate *RNA* from Rev and RRE

transfected cells, RRE-containing *RNA* was specifically detected, demonstrating the in vivo interaction between Rev and RRE (7, 28). Note that the RRE-binding domain of Rev overlaps with the...

...remains accessible on the RRE-bound Rev protein. Furthermore, RRE binding effectively masks the NLS of Rev, providing a mechanism to keep the RRE-containing *RNA* from being brought back to the nucleus by Rev (109).

Cellular Cofactors for REV/RRE Several cellular cofactors have been implicated in Rev-mediated *RNA* export. The cellular protein eIF-5A was shown to cross-link to Rev/RRE specifically in vitro, and a transdominant negative form of eIF-5A...

...and 5S rRNA use the same export pathway (72), eIF-5A may act as an adapter molecule between the RRE/Rev complex and a cellular *RNA* export pathway. Human RIP/Rab, a nucleoporin-like protein, was identified to interact with the activation domain of Rev by yeast two-hybrid screens and ...

...strain (173). The CRM-1/NES interaction is RanGTP dependent and sensitive to leptomycin B, a drug that blocks both Rev-and Rev/RRE-mediated *RNA* export (261).

Another positive cofactor in the Rev transactivation pathway is *RNA* helicase A (RHA). RHA was originally identified as a protein binding to the constitutive transport element (CTE) of type D retroviruses (see below) (239). We...

...is insensitive to leptomycin B (240). Therefore, we propose that RHA acts at a step prior to nuclear export in the Rev transactivation pathway. Since *RNA* helicases have been implicated in the release of mature mRNA from the spliceosomes (180, 182), a possible mechanism of RHA is in the premature release...

...140).

RRE-binding cellular proteins have also been identified. A novel nuclear factor with homology to heterogeneous nuclear ribonucleoprotein particle (hnRNP) F interacts with RRE *RNA* in vitro and may negatively regulate RRE-mediated gene expression (265). The generic splicing factor ASF/SF2 binds to RRE in a Rev-dependent manner...

...require the nuclear export of unspliced mRNA for the synthesis of viral structural proteins and genomic encapsidation. Instead of encoding transactivating proteins and their cognate *RNA* response elements, simple retroviruses encode cis-acting *RNA* elements that interact directly with the cellular *RNA* export ...exports via the 5S rRNA export pathway, CTE seems to utilize a distinct mRNA export pathway (72, 189, 214). More specifically, nuclear export of CTE *RNA* is independent of CRM-1 and is insensitive to leptomycin B inhibition (184).

Since CTE functions in the absence of any viral protein, it is likely that it interacts directly with components of a particular cellular export machinery by means of *RNA*-protein interaction. Two CTE-binding proteins, RHA and TAP, were recently identified (101, 239). As noted above, RHA contains a nuclear export signal that is insensitive to leptomycin B. However, whether RHA mediates the nuclear export of CTE *RNA* and its potential interaction with TAP remain to be determined.

A direct repeat (DR) sequence located between the env gene and the 3' LTR of...

...of the HIV Gag protein. RSV mutants that lack this element did not replicate well and seemed to have a significant reduction of unspliced viral *RNA* (179). Like MPMV and SRV-1, RSV does not encode a Rev-like protein, so it is likely that this cis-acting DR element also...

...promotes expression of CAT gene placed in an intron (57, 114, 116). However, the mechanism by which PRE functions to facilitate cytoplasmic appearance of unspliced *RNA* may not be restricted to direct *RNA* export (T Hope, personal communication). A 119-nucleotide *RNA* element within the herpes simplex virus thymidine kinase gene (a naturally intronless viral

gene) can also promote cytoplasmic accumulation of unspliced b-globulin transcript; this...

...binds hnRNP L (143). Additional cis-acting elements similar to PPE were also identified within the TK gene (185). Table 1 summarizes the various viral *RNA* elements for posttranscriptional regulation.

LENTIVIRUS-HOST INTERACTIONS

Infection by lentiviruses or expression of specific lentiviral gene products can have profound effects on phenotypes of the...entry has greatly propelled this area of research. Similarly, studies on the mechanism of Rev function have unraveled novel pathways for the nucleocytoplasmic transport of *RNA* and proteins. A more direct application of lentivirus research relates to the design of gene transfer vectors. The unique ability of lentiviruses to infect nondividing...

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TABLE 1 Cis-acting *RNA* elements for posttranscriptional regulation of viral gene expression

Figure 1 Genomic structure of lentiviruses. The genomic organization of several lentiviruses is depicted. Moloney leukemia virus...

...regulatory factor binding site. (Adapted from 129,246.)

Figure 4 Schematic representation of the interaction of the Tat:Cyclin T:CDK9 heterotrimeric complex with the *TAR* *RNA* element. Tat is shown interacting with the bulge region, whereas cyclin T is shown interacting primarily with the loop region. (Adapted from 50.)

Figure 5 A model for the posttranscriptional regulation of HIV-1 *RNA* . *RNA* helicase A (RHA) is recruited to the viral mRNA splicing complex by directly binding to RRE and mediates the premature release of unspliced or incompletely...

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4/3,K/12 (Item 3 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03546274 H.W. WILSON RECORD NUMBER: BGSI97046274 (USE FORMAT 7 FOR FULLTEXT)

Intracellular antibodies (intrabodies) for gene therapy of infectious diseases.

Rondon, Isaac J
Marasco, Wayne A

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TEXT:
... will then limit the spread of the virus in the patient (3). Different modalities of intracellular immunization have been developed, broadly divided into two categories: *RNA*-based and protein-based suppressors. The *RNA*-based suppressors include antisense *RNA*, ribozymes (130), and *RNA* decoys (141). Antisense *RNA* and ribozymes hybridize to target viral transcripts to inactivate them. *RNA* decoys interact and sequester regulatory proteins (e.g. Tat, Rev) that are essential for virus replication. The *RNA*-based suppressors are limited to the cytoplasmic compartment. The protein-based suppressors include transdominant mutant proteins (91), suicide molecules (14), and intracellular antibodies (96). Transdominant...AAV virus, and infection with several primary isolates from HIV-1 patients was effectively blocked in transduced T-lymphocytes (26).

TAT
Tat is a regulatory *RNA*-binding protein essential in the life cycle of HIV-1. HIV-1 encodes two forms of Tat, encoded by one (72 amino acid) or two (86 amino acid) exons. Tat protein activates transcription by association with a stable *RNA* hairpin transactivating region (*TAR*) that forms in the 5'-untranslated leader of nascent viral transcripts and promotes efficient elongation of transcription in vivo (82, 92, 97). This Tat-mediated...

...between Tat and Tat-binding cellular proteins, some of which may be transcription factors themselves (79, 111), as well as cellular proteins bound to the *TAR* region (54, 97) (Figure 5). In addition, Tat protein has other roles. Tat can easily be taken up by cells growing in tissue culture; it21). NF-kB binding to the enhancer elements in the HIV-1 LTR can cause *TAR*-independent activation of viral transcripts (11, 87). Thus, Tat is likely to have both direct and indirect effects in the pathogenesis of HIV-1.

The...

...stably inhibiting HIV-1 replication in transduced cells from HIV-1-infected patients than was sFv 105 in analogous studies.

REV
Rev is another regulatory *RNA*-binding protein essential in the life cycle of HIV-1. Rev is encoded by multiply spliced viral mRNA and appears to function efficiently only in a multimeric form (112). The Rev protein interacts with a short *RNA* sequence in the envelope gene Rev Responsive Element (RRE) to control the export of late mRNA from the nucleus; this

allows expression of virus structural the early stages of HIV-1 infection. RT specifically reverse transcribes the viral ribonucleic acid (*RNA*) to DNA, needed for integration into the host chromosome (Figure 7).

The anti-HIV-1 activity of anti-RT intrabodies has been studied by two ...

...neutralizing in assays of enzyme activity. The authors proposed that intracellular binding of anti-RT Fabs may sterically hinder movement of the enzyme along the *RNA* template or otherwise disrupt RT secondary structure.

Although most of the inhibitory effect on HIV-1 replication was observed in cell lines expressing the Fab...

...studies or virion infectivity studies.

NUCLEOCAPSID

The inner core of most retroviruses is formed by a shell of capsid protein molecules (p24) surrounding the dimeric *RNA* genome in close association with approximately 2000 molecules of nucleocapsid protein (NC), 20-50 molecules of reverse transcriptase and integrase (27), as well as tRNAs, 5S *RNA*, 7S *RNA*, and ribosomal RNAs (37). The NC protein contains two highly conserved zinc finger motifs (6). In vitro studies have shown that the NC is required for genomic *RNA* dimerization, for correct encapsidation, for annealing of the tRNA primer to its complementary binding site (PBS) on the genomic *RNA*, and for the minus-strand DNA transfer during reverse transcription (117, 121). These observations indicate that the NC plays an essential role in the afferent...sFv intrabodies.

Figure 5 Strategy for inhibition of Tat-mediated transactivation of viral gene expression by anti-Tat sFv intrabodies. Left side, Tat binding to *TAR* *RNA* and other cellular factors are required for transactivation. Right side, anti-Tat sFv binding to Tat may inhibit transactivation by inhibition of Tat nuclear import...

...of intron containing viral mRNAs. Left side, Rev binding to the cis-acting RRE sequence in unspliced and singly spliced viral mRNA leads to cytoplasmic *RNA* transport, viral structural protein synthesis, virus assembly, and production of infectious virions. Right side, anti-Rev sFv binding to any of several critical epitopes on...L, Buonaguro FM, Giraldo G, Ensoli B. 1994. The human immunodeficiency virus type 1 Tat protein transactivates tumor necrosis factor beta gene expression through a *TAR* -like structure. J. Virol. 68:2677-82

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4/3,K/13 (Item 1 from file: 266)

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Modeling *RNA*-based HIV gene therapeutics in SCID-hu mice

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SPONSORING ORG.: NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

DATES: 2001/01/02 TO 2012/31/06 FY : 2003

Modeling *RNA*-based HIV gene therapeutics in SCID-hu mice

...SUMMARY: vector transduced hematopoietic progenitor cells. Several new exciting developments occurred recently in the areas of stem cell biology, lentiviral gene transfer vectors, ribozyme targeting, and *RNA*-based therapeutics and, therefore, the stage is currently set to achieve success. In the present proposal, we would like to exploit these new technologies and...

... Project 2) are interactive and complimentary to the objectives of accompanying interactive R01 proposal (Project 1) by J. Rossi entitled "Combinatorial use of anti-HIV *RNA* -based therapeutics." The specific objectives of our proposal are: 1) Determine the effect of retrovirally transduced pol III promoter driven *nucleolar* , nuclear and cytoplasmtargeted anti-HIV nbozymes *TAR* and RBE decoys, either individually or in combination, on the lineage specific differentiation of CD34+ cells into macrophages in vitro and into thymocytes in vivo in the SCID-hu thy/liv grafts and investigate the mechanism of action of *RNA* -based therapeutics in differentiated cells. 2) Determine the in vivo protective effects of different anti-HIV-1 RNAs, individually and in combination, in SCID-hu...

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IDENTIFYING NO.: 5R01AI42552-06 AGENCY CODE: CRISP

Combinatorial use of anti-HIV *RNA*-based therapeutics

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PERFORMING ORG.: BECKMAN RESEARCH INST OF CITY OF HOPE, DUARTE, CALIFORNIA

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DATES: 2001/01/98 TO 2012/31/06 FY : 2003

Combinatorial use of anti-HIV *RNA*-based therapeutics

...SUMMARY: transcriptase and protease for the treatment of HIV-1 infection. Patients on HAART have witnessed rapid decreases in blood and tissue levels of HIV-1 *RNA* and overall positive outcomes. Despite the overall success of HAART, reservoirs of HIV-1 continue to replicate in patients on HAART, necessitating prolonged and perhaps...

... overall goal of this program is to genetically modify human cells for resistance to HIV-1 infection and replication. To this end a series of *RNA* based anti-HIV-1 agents have been developed and tested. These inhibitory RNAs target multiple steps in the viral life cycle as well as the...

...led to the development of Pol III expressed, chimeric RNAs that localize the inhibitory RNAs to the cytoplasm, nucleus or nucleolus. We have shown that *nucleolar* localization of an anti-HIV-1 ribozyme, *TAR* or RBE decoy provides potent inhibition of viral replication. By combining the composition and intracellular localization of this collection of antiviral RNAs, it should be possible to achieve long-term, synergistic inhibition of HIV-1 replication. Importantly, the use of combinatorial inhibitory *RNA* therapy in conjunction with HAART may provide additional synergy, allowing reduced dosing of HAART reagents. The overall objectives of this proposal are to test the...

... inhibitory RNAs and several HAART reagents. This program is part of an interactive R01 program (Project 1) with Dr. Ramesh Akkina (Project 2) entitled "Modeling *RNA*-based HIV gene therapeutics in the SCID-hu mouse." The Specific Aims of this proposal are: 1) construction of retroviral and lentiviral vectors harboring multiple...

... RNAs and HAART agents in blocking infectious spread of HIV-1 in cell culture; 5) transduction of primary CD34+ cells with the single and combinatorial *RNA* expressing vectors to monitor differentiation in culture and differentiation in the SCID-hu mouse model. This aim is to be carried out collaboratively with Dr...

...DESCRIPTORS: combination chemotherapy; drug interaction; gene therapy; gene expression; human tissue; immunofluorescence technique; AIDS therapy; HIV infection; disease /disorder model; multidrug resistance; in situ hybridization; virus *RNA*; zidovudine; *RNA* directed DNA polymerase; nonhuman therapy evaluation; tissue /cell culture; virus replication; human immunodeficiency virus 1; northern blotting; ribozyme; transfection /expression vector; SCID mouse; CD34 molecule

4/3,K/15 (Item 3 from file: 266)
DIALOG(R)File 266:FEDRIP

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IDENTIFYING NO.: 2R37AI29329-14 AGENCY CODE: CRISP
Enhancing the Intracellular Functioning of HIV Ribozymes
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DATES: 2007/03/96 TO 2006/30/07 FY : 2002

...SUMMARY: in and demonstrations of efficacy both in vitro and in vivo have taken place. Ribozymes have the attributes of site-specific cleavage of the target *RNA*, the potential for turning over multiple substrates, and general lack of toxicity since they do not encode proteins In order to attain the goal of using anti-HIV ribozymes for treatment of HIV infection, we have been exploring several important areas of ribozyme function. These include identifying optimal *RNA* target sites for ribozyme interaction, co-localization of ribozyme and HIV RNAs and optimizing ribozyme expression from the backbones of lentiviral and retroviral vectors. The...
... analyses of engraftment potential. This latter work will be carried out collaboratively with Dr. Bruce Torbett at the Scripps Research Institute. 2) Functional analysis of *nucleolar* trafficking of HIV RNAs and regulatory proteins and in vivo selection for anti-HIV *nucleolar* localized ribozymes. A *nucleolar* localized anti-HIV-1 ribozyme as well as *nucleolar* localized *TAR* an RJ3E decoys have provided potent inhibition of HIV-1 infection in cultured cells. We will extend our analyses of these constructs to primary CD34+ cells and T-lymphocytes as part of specific aim 1. In aim 2 further analyses of the functional role of HIV-1 *RNA* trafficking through the nucleolus will be explored. Specifically, we will investigate the possibility that 2'0-methyl covalent backbone modifications in the R region of HN-1 *RNA* are guided by a cellular small *nucleolar* *RNA*. We will engineer a small *nucleolar* *RNA* to misdirect 2'0 methylations to specific sites of protein interaction in the HIV *TAR* and R13E elements. Finally, an in vivo SELEX scheme will be developec to select for new targets, both in HIV-1 and cellular RNAs using a randomized binding arm library of *nucleolar* localized ribozymes. The results of the work proposed in this aim should extend our knowledge of the functional role of HIV *RNA* and protein trafficking through the nucleolus, and provide new ribozymes and other inhibitory RNAs for inhibition of HIV-1 infection. The overall objective of this work is tc enhance our understanding of HIV *RNA* localization via the use of ribozymes, and to identify the best ribozyme-HIV target strategies for future use in human gene therapy.

4/3,K/16 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0295759 DBR Accession No.: 2002-17606
A *nucleolar* *TAR* decoy inhibitor of HIV-1 replication - vector-mediated gene transfer and expression in host cell and polymerase chain reaction for use in HIV virus-1 gene therapy
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ISSN: 0027-8424 CODEN: 0027-8424; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AME; (2002) 99, 22, 14047-14052
LANGUAGE: English

A *nucleolar* *TAR* decoy inhibitor of HIV-1 replication - vector-mediated gene transfer and expression in host cell and polymerase chain reaction for use in HIV virus-1...
...ABSTRACT: critical regulatory factor in HIV-1 gene expression. It mediates the transactivation of transcription from the HIV-1 LTR by binding to the transactivation response (*TAR*) element in a complex with cyclin T1. Because of its critical and early role in HIV gene expression, Tat and its interaction with the *TAR* element constitute important therapeutic targets for the treatment of HIV-1 infection. Based on the known *nucleolar* localization properties of Tat, we constructed a chimeric small *nucleolar* *RNA*-*TAR* decoy thatlocalizes to the nucleoli of human cells and colocalizes in the nucleolus with a Tat-enhanced GFP fusion protein. When the chimeric *RNA* was stably expressed in human T lymphoblastoid CEM cells it potently inhibited HIV-1 replication. These results demonstrate that the *nucleolar* trafficking of Tat iscritical for HIV-1 replication and suggests a role for the nucleolus in HIV-1 viral replication. (6 pages)
DESCRIPTORS: *nucleolar* *RNA*-transactivation response decoy-inhibitor, TAT-enhanced green fluorescent protein prep., vector-mediated gene transfer, expression in host cell, polymerase chain reaction, appl. HIV virus-1...

4/3,K/17 (Item 1 from file: 149)

01421140 SUPPLIER NUMBER: 14003767 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Molecular targets of gene transfer therapy for HIV infection.
Buchschacher, Gary L., Jr.
JAMA, The Journal of the American Medical Association, v269, n22, p2880(7) June 9,
1993
PUBLICATION FORMAT: Magazine/Journal ISSN: 0098-7484 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional
WORD COUNT: 7338 LINE COUNT: 00611

... target specific aspects of HIV replication, basic aspects of the HIV replication cyde will be reviewed briefly.

HIV AND THE RETROVIRUS REPLICATION CYCLE

Retroviruses are *RNA* viruses that replicate through a DNA intermediate. Virions contain two identical, single-stranded *RNA* molecules of positive polarity surrounded by the viral nucleocapsid protein. The remaining core of the virus is composed of the capsid and matrix proteins. In...

...expression from the HIV LTR.(6) Tat mediates this effect by interacting with a segment of the R region in the 5' LTR, termed the "*TAR* element" (for trans-activating response), of transcripts initiated from the promoter.(7) The *TAR* element has been shown to form a stable stem loop structure.(8) Tat, along with unidentified cellular protein(s),(9,10) interacts with the *RNA* stem loop and apparently prevents premature termination of initiated transcripts.(11) Additional mechanisms of action,

such as increasing the translation efficiency of transcripts, also have...

...of full-length and singly spliced transcripts.(13,14) As with the Tat protein, Rev mediates its effect by interacting with a segment of transcribed *RNA*. The target of Rev is a segment of *RNA*, termed the rre (for Rev-responsive element), that is contained within the env gene. The rre has extensive secondary structure; interaction of Rev with the...

...in a pH-independent manner.(19,20) Following the release of the viral nucleic acid into the cytoplasm by an unknown mechanism, the single-stranded *RNA* molecules serve as a template for reverse transcriptase to generate a double-stranded DNA copy of the genome.(21,22)

The DNA copy of the...of viral structural proteins and replication enzymes. Full-length transcripts are incorporated into virion cores, probably through the interaction of capsid proteins with segments of *RNA* known to be important for encapsidation of the viral *RNA* into virions.(25'27) As virions bud from cells, they obtain their viral glycoprotein-containing envelopes. Released particles are then able to initiate a new...of this strategy, currently the titer of vector virus produced is far too low to be of practical use. Alternatively, isolation of the cis-acting *TAR* element or rre and their placement on a heterologous vector (eg, a murine retrovirus vector) can be used to limit gene expression to HIV-infected...

...with one or more steps in HIV replication but do not result in the death of the infected cells. These include, for example, viral-specific *RNA* ribozymes(39) or dominant negative mutants of viral gene products.

Elimination of Infected Ceils

In an effort to stimulate a more effective immune response against...

...inhibition of protein synthesis. Initial experiments used the luciferase gene (luc) as a marker for the DT-A gene expressed from a construct containing the *TAR* element.38 Although gene expression was induced in the presence of Tat, the results indicated that, for these genes, additional control of gene expression might...in the sequestering of cellular or viral components used in virus repilcation. An example of this is the use of high levels of expression of *TAR* sequences, which are known to interact with Tat and an unknown cellular factor. A chimeric tRNA-*TAR* construct, which was expressed in a T-cell line at high levels using a doublecopy murine retroviral vector, has been used to interfere with HIV replication.(47) Expression of high levels of *TAR* element sequences did not appear to affect cell viability. Further studies indicated that the overexpressed *TAR* elements were acting as decoys for a Tat-transactivation protein complex.(48) Another group used a Tat-inducible construct to express multimerized *TAR* elements. They found that induced transcription of these *TAR* sequences could decrease expression of a marker gene expressed from the HIV LTR.(49) Using a similar strategy, rre sequences were overexpressed in experiments using a T-cell line.(50) When challenged with HIV, these cells inhibited replication, though not as efficiently as cells expressing the *TAR* elements.

Antisense *RNA* molecules (sequences complementary to viral *RNA* sequences) also have been investigated as possible antiviral reagents. In this approach, it is hoped that the antisense molecules will hybridize to viral transcripts and thereby disrupt replication, translation, or encapsidation into virion particles ofviral *RNA* molecules. In one study, antisense *RNA* corresponding to the HIV-1 primer binding site or to a region just 5' to the primer binding site was used to interfere with HIV replication in a CD4-positive lymphocyte-derived cell line.(51) Antisense *RNA* directed against tat and rev sequences also has been used to inhibit HIV replication.(52) After a T-lymphocyte line expressing antisense tat *RNA* molecules was infected with HIV, viral inhibition was observed for 2 weeks when a high multiplicity of infection was used. When a low multiplicity was used, however, inhibition continued for at least 5 weeks. Another group also found antisense *RNA* overlapping the tat gene initiation codon to be inhibitory, but again the effect was observed to be transient.(53)

Recently, an adeno-associated virus vector was used to introduce an

antisense sequence into both human hematopoietic and nonhematopoietic cell lines.(54 The antisense *RNA* used was an LTR sequence corresponding to the *TAR* element in the 5' LTR and part of the polyadenylation signal in the 3' LTR. Antisense *RNA* was produced constitutively and did not result in any apparent toxicity. When challenged with HIV, cells containing the antisense construct contained less viral mRNA, suggesting *RNA* hybrids were being rapidly degraded. Human immunodeficiency virus replication was inhibited up to 94% to 98% for 20 days as measured by a reverse transcriptase...

...99%. If these promising results can be extended to primary cells, this strategy may be effective for slowing HIV spread in vivo.

Ribozymes are small *RNA* molecules capable of catalyzing cleavage of specific *RNA* sequences. The use of ribozymes that target HIV sequences and that therefore might destroy HIV *RNA* before it can be replicated, translated, or packaged has been investigated. In one study,(55) a ribozyme specific for gag transcripts was expressed in CD4-positive HeLa cells. Constitutive expression of the ribozyme was found to decrease virus replication as assayed by viral antigen (p24) and *RNA* levels when these cells were challenged with HIV. Other attempts to use ribozymes specific for U5 sequences (56) or other 5' leader sequences(57, 58) also resulted in a varying detectible decrease in HIV replication. In addition, a ribozyme directed against HIV-1 integrase gene *RNA*, when expressed in Escherichia coli, has been shown to cleave the target sequence and to prevent integrase gene expression? Further work designed to identify more...

...be in considerable molar excess for the effect to be maximal. It has been suggested that the mutant Tat inhibited wild-type Tat by preventing *nucleolar* localization, possibly through the formation of inactive heterodimers.

A Rev routant that interferes with wild-type Rev function also has been identified.(64) The mechanism...initiation and stabilizes elongation.

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4/3,K/18 (Item 2 from file: 149)

01313399 SUPPLIER NUMBER: 11517856 (USE FORMAT 7 OR 9 FOR FULL TEXT)

The AIDS virus: what we know and what we can do about it.

Wong-Staal, Flossie
The Western Journal of Medicine, v155, n5, p481(7)
Nov,
1991
PUBLICATION FORMAT: Magazine/Journal ISSN: 0093-0415 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional
WORD COUNT: 4527 LINE COUNT: 00463

...ABSTRACT: as a discussion of how different viral characteristics might be exploited for therapy. HIV is a retrovirus, which means that the viral genes consist of *RNA*. Unlike some *RNA* viruses, however, the retrovirus must make a DNA copy of the genes before it can replicate. (This ''backward'' synthesis of DNA from *RNA* is the source of the ''retro'' nomenclature.) This DNA copy of the viral genes is actually integrated directly into the chromosomes of the human host...

...replication or halting its spread. Halting the replication of the virus may focus on the viral enzyme known as reverse transcriptase, which transcribes the viral *RNA* into DNA. This approach benefits by the fact that no such enzyme exists in human cells under normal circumstances, and therefore a drug might inhibit...

... repeat (LTR). Infection begins with the binding of the viral

envelop to the cellular receptor, which then allows entry of the viral core containing the *RNA* genetic information. A viral enzyme, reverse transcriptase, converts the *RNA* to DNA provirus, which can then move to the nucleus, where it is integrated into the host chromosomal DNA, catalyzed by another viral enzyme, integrase...

...host chromosome, the infection is established and cannot be cured; only viral expression can be suppressed. The integrated provirus serves as a template for messenger *RNA* (mRNA) that can then be processed and translated into precursor proteins. The proteins then assemble and incorporate the genomic *RNA* to form virion particles. The maturation of these particles requires processing of the precursor proteins, using yet another viral enzyme, protease.

Every step in this...nucleus. The Rev protein, by interacting with a specific target sequence contained in the intron of the viral precursor mRNA, is able to disengage the *RNA* from the spliceosome and facilitate export into the cytoplasm (Figure 3). By doing so, Rev actually diversts the distribution of multiply spliced *RNA* to unspliced or less spliced forms, so that Rev functions at the expense of its own production and the production of the other regulatory proteins...
...to this protein, all of them resulting in the activation of gene expression from the viral LTR. The target sequence that tat recognizes is called *TAR*, located immediately downstream of the site of transcription initiation. The tat gene has been known to increase the frequency of transcriptional initiation and to act...

...mechanisms, tat and rev also have several parallel features. They are both small nucleoproteins that are found predominantly in the nucleolus. Both of them recognize *RNA* rather than DNA targets, a feature that distinguishes them from most other viral transactivators. The target for rev is called the rev response element, or RRE, and the target for tat is *TAR*. In both cases, the *RNA* targets are highly structured molecules, and the recognition of them by the viral transactivators is largely structural. The rev gene product binds to a specific "hammerhead" (stem-loop II) region of the RRE, and tat binds to three unpaired bases in the so-called bulge of *TAR*. That binding of these viral transactivators to their target RNAs is not sufficient for function and that cellular factors are also involved are evident because...

...may not be functional. For example, the domain for binding to RRE has been mapped on the Rev protein. This region also doubles as the *nucleolar* targeting domain. In addition, there is another critical domain in which mutations do not affect the capability of the protein to bind to RRE, and ...

...that this region is an activator domain that mediates interaction of the Rev protein with cellular factor(s). Essential functional domains including the regions for *RNA* binding ...also what its role is in eukaryotic gene regulation.

Similarly, not one but several cellular factors have been shown to bind to either Tat or *TAR*. For example, a 68-kilodalton (kd) protein that recognizes the loop sequence of the *TAR* region has been described. A 45-kd protein with the same *TAR*-binding specificity is prevalent in T cells. A similar-sized protein binds specifically to the stem between the loop and the bulge (Figure 4). Again...

...by expression of the core protein p24 or viral reverse transcriptase. The sense-strand of the same sequence had no effect. When the expression of *RNA* is examined in the antisense-treated cells, neither tat nor rev is completely inhibited--that is, some *RNA*, including singly spliced viral *RNA*, is expressed. The unspliced genomic *RNA*, however (the gag-pol message), appears to be highly sensitive to rev depletion and is completely repressed. As a result, viral production is also repressed...
...gene an attractive target for virus inhibition.

As discussed earlier, both tat and rev transactivations involve a number of players: the viral protein, a viral *RNA* element, and cellular factors, each interacting with the others. It is possible to devise means

of interrupting those interactions that are crucial to their functions. For example, some mutant proteins are negative dominant. These proteins can retain binding activity to their *RNA* targets but are not able to interact with the proper cellular factors and therefore would not function. They can thus act as competitive inhibitors of the wild-type protein. Alternatively, oligonucleotides that can hybridize to the stem-loop sequences of the *RNA* target can presumably disrupt the structure or the folding of these molecules, thereby rendering them incapable of interacting with both viral and cellular factors. Synthetic...Klotman, S. Daefler, F. Wong-Staal, unpublished data, 1991).

There are many variations on this theme. One example is the expression of multiple copies of *TAR* *RNA* as a decoy to compete with normal *TAR* in the virus genome for expression. A potential problem of expressing a *TAR* or RRE decoy, however, is that it may also sequester cellular factors that may be important for cell function. To overcome this problem, a vector has been devised in which the expression of *TAR* is conditional on tat acting on its promoter, which is the HIV LTR. Therefore, these decoy molecules would only be expressed in infected cells, where the first sign of the expression of tat can then unleash a whole army of these *TAR* decoys to limit the extent of viral replication (J. Lisziewicz, MD, written communication, April 1991).

The foregoing discussion only relates to laboratory successes on inhibiting...

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DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01311360 SUPPLIER NUMBER: 10873149 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Arginine-mediated *RNA* recognition: the arginine fork.
Calnan, Barbara J.; Tidor, Bruce; Biancalana, Sara; Hudson, Derek; Frankel, Alan D.
Science, v252, n5009, p1167(5)
May 24,
1991
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
WORD COUNT: 3418 LINE COUNT: 00325

Arginine-mediated *RNA* recognition: the arginine fork.

TEXT:

...ARE important for many regulatory processes, but little is known about the details of sequence-specific recognition. From what is known, it appears that both *RNA* structure and nucleotide sequence function in recognition. The crystal structure of the glutaminyl tRNA synthetase-tRNA complex [1] ha shown that specific contacts are made between amino acid side chains and bases in non-base paired regions of the *RNA*, while studies of the R17 coat protein [2] have suggested that the overall three-dimensional *RNA* conformation contributes substantially to recognition. Recently, an arginine-rich *RNA*-binding motif has been identified in several *RNA*-binding proteins [3], including the human immunodeficiency virus (HIV) Tat protein. Peptides that contain this region of Tat bind specifically to an *RNA* stem-loop structure named *TAR* [4, 5], which is located in the HIV long terminal repeat, and *RNA* binding is essential for Tat-dependent transcriptional activation [5]. The overall charge density of the Tat peptides is important for binding, however, the amino acid sequence requirements are flexible; the sequence can be scrambled and still bind specifically to *TAR* [5].

The basic *RNA*-binding region of Tat, RKKRRQRRR (residues 49 to 57), is nine amino acids long and contains a glutamine at position 54 that is not essential for binding or activity [5]. Because it is known that a high positive charge density is important for *RNA* binding, we synthesized [6] two peptides, [R.sup.9], which contains a stretch of nine adjacent arginines (with a tyrosine at the [NH.sub.2...

...the COOH-terminus), and [K.sub.9], which contains a stretch of nine lysines (and a surrounding tyrosine and alanine), and measured their binding to *TAR* *RNA* [7]. The [R.sub.9] peptide bound to *TAR* *RNA* with the same affinity as the wild-type Tat peptide and with ten-fold higher affinity than [K.sub.9] (Fig. 1). The specificity of [R.sub.9] binding to *TAR* was identical to th wild-type peptide, whereas [K.sub.9] binding was nonspecific [7]. Because *RNA* binding of Tat peptides correlates with Tat's function as a transcriptional activator [5], we asked whether [R.sub.9] or [K.sub.9] could...

...and was 100-fold more active than the [K.sub.9]-containing protein (Fig. 2). These results confirm that transactivation of transcription correlates with peptide *RNA* binding and suggest that arginine residues are important for specific *RNA* recognition.

The low level of transactivation achieved by the [K.sub.9]-containing protein allowed us to systematically replace lysines with arginines in order to...

...52 to 53 restored transactivation to wild-type levels (Fig. 3). Electrophoretic mobility shift experiments with YKKKRKKKKKA (R52) or with YKKKKRKKKKA (R53) peptides showed that *RNA*-binding affinity and specificity were also restored to wild-type levels by the addition of one arginine at either position 52 or 53 [10]. To...

...basic region (Fig. 4). Thus, a single arginine surrounded by three to four basic amino acids on each side is sufficient for specific recognition of *TAR* *RNA*.

These and other results [4, 5, 11] clearly suggested that *RNA* structure is important in Tat-*TAR* recognition. In seemed plausible that the *RNA* backbone might be adopting a highly defined conformation and that a specific configuration of phosphates was being recognized by arginine. To identify phosphates involved in...

...contacts two adjacent phosphates (even in the wild-type peptide, which contains multiple arginines), although we cannot rule out the possibility that ethylation alters the *RNA* structure and indirectly interferes with binding. Ethylation of some phosphates, particularly the phosphate between G26 and A27, seems to enhance binding (Fig. 5), possibly by stabilizing the *RNA* structure. The minimal interference observed with lysines or with nonspecific arginines suggests that these residues may make weaker contacts with the *RNA* or that alternative phosphates may be contacted when one is modified.

How does a single arginine recognize *TAR* *RNA*? Arginine contains two terminal amino ([NH.sub.2]) groups at the [eta] position and a secondary amine (NH) at the [epsilon] position, each of which...

...and geometries are shown in Fig. 6A. Clearly, an arginine side chain can form many possible hydrogen bonds with appropriately positioned acceptor groups on the *RNA*. These acceptors can include phosphate oxygens, the ribose 2' OH, and groups on the bases (for example, O-6 and N-7 on guanine or...

...the planar geometry of the arginine amino groups. Our ethylation interference data indicate that a single arginine in Tat contacts two adjacent phosphates at the *TAR* bulge, suggesting that the phosphate backbone adopts a defined conformation that can be bridged by arginine in a fork-like arrangement. To determine a plausible...phosphorus atoms). We define the arginine fork as an interaction between a single arginine and a pair of adjacent phosphates, which mediates specific recognition of *RNA* structure. Other arginine-phosphate arrangements are possible (for example, see legend to Fig. 6B), and arginine forks with additional H-bonds are possible (for example, with a specific base or a 2' OH).

To determine whether such phosphate arrangements are found in *RNA* structures, the modeled phosphate coordinates from Fig. 6B were superimposed on all phosphate pairs in tRNA crystal structures [14]. The results indicate that double-stranded A-form *RNA* cannot readily accommodate this arrangement; the P-P distance in the model (7.1

[angstroms]) is longer than the P-P distance in A-form *RNA* (5.6 [angstroms]), and the phosphate oxygens in A-form *RNA* are not properly oriented to form H-bonds between a single arginine and a pair of adjacent phosphates. Reasonable H-bonding arrangements are much more likely to be found at discontinuous regions of *RNA*, for example, at junctions between double-stranded A-form *RNA* and a bulge or loop. The two critical phosphates in *TAR* are located precisely at the junction of the double-stranded stem and the 3-nucleotide bulge.

The cocrystal structure of glutaminyl tRNA synthetase-tRNA shows...

...additional H-bond with a ribose 2' OH. It is plausible that the arginine in Tat also interacts with a 2' OH, thus discriminating between *RNA* and DNA [4]. Although we cannot rule out base-specific contacts, for example, with an essential uridine in the bulge [4, 5, 11], it seems...

...contacts between one arginine and a highly oriented pair of phosphates can account for the modest 10- to 20-fold specificity of Tat binding to *TAR* [4, 5, 11]. The interactions that stabilize the overall folding of *TAR* and the orientation of these particular phosphates remain to be determined. In addition to the specific arginine contact, the charge density of the basic region...

...Tat is important for binding [5] and may provide a nonspecific electrostatic scaffold to help orient the arginine. Tat is perhaps the simplest example of *RNA* recognition in that a single amino acid interacts with a single feature of the *RNA*; other proteins may achieve higher specificity through multiple arginine-*RNA* or other interactions. In the case of Tat, although *RNA* binding is essential for transactivation, the modest specificity for *TAR* is insufficient to account for the high specificity of Tat function. Other interactions of Tat, perhaps with cellular proteins, are likely to be required.

The recognition of *TAR* by Tat highlights fundamental differences between *RNA* recognition and DNA recognition. It is clear from the structures of protein-DNA complexes that sequence-specific discrimination derives primarily from direct base-specific contacts...

...commonly made in the DNA major groove [5]. In most cases, DNA tertiary structure does not seem to be of major importance in recognition. That *RNA* recognition often seems to rely on *RNA* tertiary structure is emphasized by the finding that the Tat-*TAR* interaction uses only a single arginine side chain in the midst of an apparently unstructured segment of basic amino acids [5] to recognize a specific backbone conformation of *TAR*. The unstructured nature of the unbound polypeptide is supported by the fact that the sequence of the Tat basic region can be simplified to a single arginine embedded in a set of eight lysines. The peptide conformation when bound to *TAR* remains to be determined. Specific recognition of *TAR* appears to occur by indirect readout of the base sequence and direct contact with the phosphate backbone, and may not involve any base-specific contacts. Differences between DNA and *RNA* recognition are also apparent in studies of TFIIIA, a zinc finger--containing protein that binds to the same site on both DNA and *RNA*. DNA recognition seems to occur through base-specific contacts in the major groove; *RNA* recognition appears to be primarily backbone structure-specific [16].

Other *RNA* hairpins and bulges form stable, ordered tertiary structures [17], further emphasizing that *RNA* structure can provide information for protein-*RNA* complex formation. The structure of an *RNA* pseudoknot reveals that phosphates can be arrayed in unusual, electrostatically unfavorable geometries through tertiary *RNA* interactions [18]; binding of basic amino acids to these phosphates might help to stabilize a more favorable *RNA* conformation that, in turn, would provide favorable energy for the protein-*RNA* interaction. This could explain why interaction of HIV Tat or Rev with *RNA* causes a change in *RNA* conformation upon binding [5, 19].

Other *RNA*-protein interactions will likely follow some of the principles outlined here. Arginine-rich motifs similar to the basic region of Tat are found in several *RNA*-binding proteins, including bacterial

antiterminators, ribosomal proteins, and HIV Rev [3]. Other *RNA*-binding proteins may bring basic amino acids together through protein tertiary structure rather than primary sequence and may position specific arginines to interact with defined *RNA* structures. For example, the U1 A protein, which contains a ribonuclear protein (RNP) *RNA*-binding motif, has a highly defined structure with a cluster of basic amino acids at one end and at least one arginine that is essential...

...recognition [20]. It is not yet known if this arginine makes a base-specific or structure-specific contact. For TFIIIA, the strongest interactions with 5S *RNA* are localized to junctions between stems and loops [21], similar to the stem-bulge junction in *TAR*; perhaps arginines participate in some of these interactions. Arginine has also been shown to bind to the guanosine binding site of a group I intron [22]; however, this interaction involves H-bonding to a guanine base in the *RNA* and is distinct from the arginine fork proposed here. Many *RNA*-binding proteins, including heterogeneous nuclear *RNA*-binding proteins and *nucleolar* proteins, contain clusters of methylated arginines, most commonly [N.sup.G],[N.sup.G]-dimethylarginine [23]. Because methylation would block H-bonding but would not alter the charge of the side chain, arginine methylation could provide a mechanism to regulate *RNA* binding between specific and nonspecific modes. While it is clear the *RNA* recognition will involve more than just arginine forks, it seems reasonable to suggest that arginine-mediated recognition of *RNA* structure may be an important part of many *RNA*-protein complexes.

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...spectrometry (University of California, Berkeley), and purity and concentrations were confirmed by native polyacrylamide (20%) gel electrophoresis in sodium acetate, pH 4.5 (30mM).

[7] *TAR* RNAs were transcribed by T7 *RNA* polymerase in vitro with synthetic oligonucleotide templates [5] [J. F. Milligan and O. C. Uhlenbeck, Methods Enzymol. 180, 51 (1989)]. All RNAs contained GG at their 5' end, which increased the efficiency of transcription, and CC at the 3' end to base pair with GG. Wild-type *TAR* *RNA* contained sequences +18 to +44 of the HIV long terminal repeat *TAR* site. Mutant TARs were also synthesized and specificity of binding of each peptide was determined [5]. All RNAs were purified on 10% polyacrylamide/8 M...

...in ammonium acetate (0.5 M), magnesium acetate (10 mM), EDTA (1 mM), and SDS (0.1%), extracted twice with phenol, and ethanol-precipitated. Purified *RNA* was resuspended in sterile deionized water. The concentrations of labeled RNAs were determined from the specific activitiy [sub.32.P]CTP incorporated into the transcripts. Unlabeled *RNA* was quantitated by spectrophotometry. *RNA* electrophoretic mobility shift assays were performed as described [5]. Briefly, peptide and *RNA* were incubated together for 10 min on ice in binding reactions (10 [microliter]) that contained tris-HCl pH 7.5 (10 mM), NaCl (70 mM), EDTA (0.2 mM), and glycerol (5%). Peptide-*RNA* complexes were resolved on polyacrylamide (10%) gels in tris-borate-EDTA [0.5 X TBE; in Current Protocols in Molecular Biology, F. M. Ausubel et...

...U. Delling, C.-H. Chen, C. A. Rosen, N. Sonenberg, Genes Dev. 4, 1365 (1990); C. Dingwall et al., EMBO J. 9, 4145 (1990).

[12] *TAR* *RNA* (31 nucleotides) was labeled with [sub.32.P] at the 5' end with T4 polynucleotide kinase. *RNA* phosphates were ethylated with ethylnitrosourea under denaturing conditions as described [V. V. Vlassov, R. Giege, J.-P. Ebel, Eur. J. Biochem, 119, 51 (1981)], except...

...using a saturated solution of ethylnitrosourea in ethanol) and tRNA (2 [microgram], and wee incubated at 80 [degrees] C for 5 min. After modification, the *RNA* was ethanol -precipitated, washed with 100% ethanol and lyophilized. Peptides were bound to the modified *RNA* (500,000 cpm) at concentrations that gave < 50% binding (specific) by gel shift [7] or at

higher concentrations that gave nonspecific binding. Free and bound RNAs were visualized by autoradiography. The bands were excised and the *RNA* was eluted from the gel [7], ethanol precipitated with yeast tRNA (20 [microgram]), and lyophilized. To cleave the phosphotriester bonds (at the modified phosphates), samples...

...i+2), and those distant in primary sequence but near each other in the tertiary structure. In no case did phosphate pairs in double-stranded *RNA* match the template. The (i, i+1) pattern frequently appeared surrounding the first or last unpaired base in a bulge or loop, and the (i...

...DESCRIPTORS: *RNA*--

4/3,K/20 (Item 1 from file: 159)
DIALOG(R)File 159:Cancerlit
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01915587 PMID: 93686277
HIV-1 transactivator.
No affiliation given
Harvard AIDS Inst Ser 1991, 1 p43-310,
Document Type: MONOGRAPH
Languages: ENGLISH
Main Citation Owner: NOTNLM
Record type: Completed

...viral regulatory proteins tat and rev, are expressed, whereas, late in infection production switches to full-length, unspliced transcripts that act both as the virion *RNA* and the mRNA for the gag-pol polyprotein. HIV-1 transactivator is reviewed in the following chapters: *RNA* interactions of the tat and rev proteins of HIV-1; the role of the *TAR* region and tat protein in HIV-directed gene expression; *RNA*-protein interactions required for regulation of HIV gene expression; cellular factors involved in regulating HIV gene expression; biochemical characterization of REV-RRE interaction; analysis of...

... the rex protein of HTLV-1; tat-mediated trans-activation as a presplicing event requiring a functional HIV-1 TATAA element; analysis of the TAT/*TAR* interaction in Xenopus oocytes; transcriptional regulation of HIV; functional topography of lentivirus tat proteins defined by domain switching; *nucleolar* targeting singles of HIV; characterization of the specific interaction between TAT and *TAR* *RNA* ; and the exogenous trans-activation activity of HIV-1 tat.
Chemical Name: Gene Products, rev; Gene Products, rex; Gene Products, tat ; *RNA*, Viral; Trans-Activators; Viral Proteins

4/3,K/21 (Item 1 from file: 444)
DIALOG(R)File 444:New England Journal of Med.
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Medical Progress: Nonmelanoma Cancers Of The Skin (Review Article)

Preston, Diana; Stern, Robert.
The New England Journal of Medicine
Dec 3, 1992; 327 (23),pp 1649-1662
LINE COUNT: 00688 WORD COUNT: 09497

TEXT
...58-74). In 1775, Sir Percival Pott reported an increased risk of squamous-cell carcinoma of the genitals in English chimney sweeps exposed to coal-*tar* products, the first recognized example of a chemical carcinogen (Ref. 75). Numerous chemicals are associated with an increased

risk of nonmelanoma skin cancer, primarily squamous...deeper tumors (Ref. 153-155). A fluorinated pyrimidine, fluorouracil inhibits the methylation of deoxyuridylic acid to thymidylic acid, interfering with the synthesis of DNA and *RNA* and cell growth (Ref. 156). Available as creams and solutions, fluorouracil is applied twice

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4/3,K/22 (Item 2 from file: 444)

00108320

Mechanisms of Disease: The Molecular Biology Of Human Immunodeficiency Virus Type 1 Infection (Review Article)

Greene, Warner C.
The New England Journal of Medicine
Jan 31, 1991; 324 (5),pp 308-317
LINE COUNT: 00566 WORD COUNT: 07820

TEXT

...whereas the pol gene gives rise to the viral reverse transcriptase and other enzymatic activities (Fig. 1). HIV-1, however, contains in its 9-kilobase *RNA* genome not only these three essential genes but also at least six additional genes (vif, vpu, vpr, tat, rev, and nef) (Fig. 1). It is...

...3' long terminal repeats (LTRs) containing regulatory sequences recognized by various host transcription factors are also depicted, and the positions of the Tat and Rev *RNA* response elements (*TAR* transactivation response] element and Rev response element) are indicated *. **FIGURE OMITTED...inner surface of the lipid bilayer and probably stabilizes the exterior and interior components of the virion. The p7 protein binds directly to the genomic *RNA* through a zinc-finger structural motif and together with p9 forms the nucleoid core. Importantly, this retroviral core also contains two copies of the single-stranded HIV-1 genomic *RNA* that is associated with the various preformed viral enzymes, including the reverse transcriptase, integrase, and protease (Fig. 2). *Figure 2.-Schematic Diagram of the HIV...

...and gp41(sup env)) and nucleocapsid (p24(sup gag), p17(sup gag), p9(sup gag), and p7(sup gag)) is identified. In addition, the diploid *RNA* genome is shown associated with reverse transcriptase, an *RNA*-dependent DNA polymerase *. **FIGURE OMITTED...

...the entry of HIV-1 into the cell (lck denotes a lymphoid-specific tyrosine kinase that binds to CD4). After uncoating, reverse transcription of viral *RNA* begins, resulting in the production of the double-stranded DNA form of the virus in the presence of the appropriate host factors. In turn, the...for the replicative phase of its life cycle (Ref. 6). Viral replication begins with the generation of a first-strand DNA copy of the viral *RNA* mediated by the HIV-1-encoded reverse transcriptase (Fig. 3). Second-strand DNA synthesis is also controlled by the reverse transcriptase but proceeds only after the action of a second pol-gene product, ribonuclease H, which partially degrades the original *RNA* template. When complete, the reverse-transcription reaction yields a double-stranded DNA replica of the original *RNA* genome containing tandem long terminal repeats at each end of the DNA in lieu of the short terminal repeats